

Rational Design, Synthesis and Biological Evaluation of Heterocyclic Quinolones Targeting the respiratory chain of *Mycobacterium tuberculosis*

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ABSTRACT

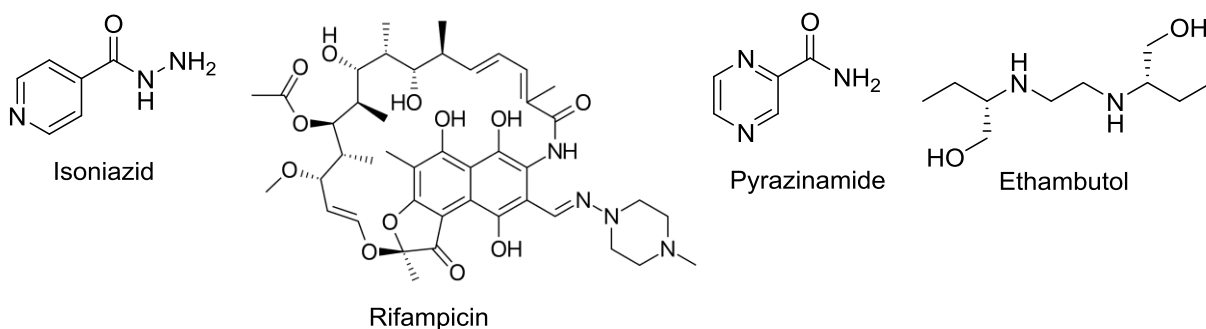
A High-throughput screen (HTS) was undertaken against the respiratory chain dehydrogenase component, NADH:menaquinone oxidoreductase (Ndh) of *Mycobacterium tuberculosis* (Mtb). 11,000 compounds were selected for the HTS based on the known phenothiazine Ndh inhibitors, trifluoperazine and thioridazine. Combined HTS (11,000 compounds) and in-house screening of a limited number of quinolones (50 compounds) identified ~100 hits and four distinct chemotypes, the most promising of which contained the quinolone core. Subsequent Mtb screening of the complete in-house quinolone library (350 compounds) identified a further ~90 hits across three quinolone sub-templates. Quinolones containing the amine based side chain were selected as the pharmacophore for further modification, resulting in metabolically stable quinolones effective against multi drug resistant (MDR) Mtb. The lead compound, MTC420 displays acceptable anti-tuberculosis activity (Mtb IC_{50} = 525 nM, Mtb Wayne IC_{50} = 76 nM and MDR Mtb patient isolates IC_{50} = 140 nM) and favourable pharmacokinetic and toxicological profiles.

INTRODUCTION

In 2014 tuberculosis (TB) globally infected 9.6 million people resulting in an estimated 1.5 million deaths.¹ With the emergence of multi-drug resistant (MDR) and extensively drug resistant (XDR) TB the need for new drug treatments targeting the disease has never been greater.² Current first line drugs for TB were developed in 1952-1966 (Figure 1). Shortcomings of these drugs include; (i) long treatment regimens (6 to 9 months) leading to patient non-compliance, (ii) adverse drug-drug interactions with anti HIV drugs (HIV/AIDS is a common co-infection) and (iii) limited or

no activity against MDR and XDR *Mycobacterium tuberculosis* (Mtb).³ Bedaquiline^{4, 5} and delamanid^{6, 7} are the only recently FDA approved drugs for the treatment of TB and their approval is currently only for MDR in cases where established treatments have failed (Figure 1).⁸ In order to find an effective treatment for MDR and XDR it is believed that a drug with a novel mode of action is required in order to circumvent resistance.

Current first line drugs used to treat TB:



Recently approved drugs to treat MDR TB:

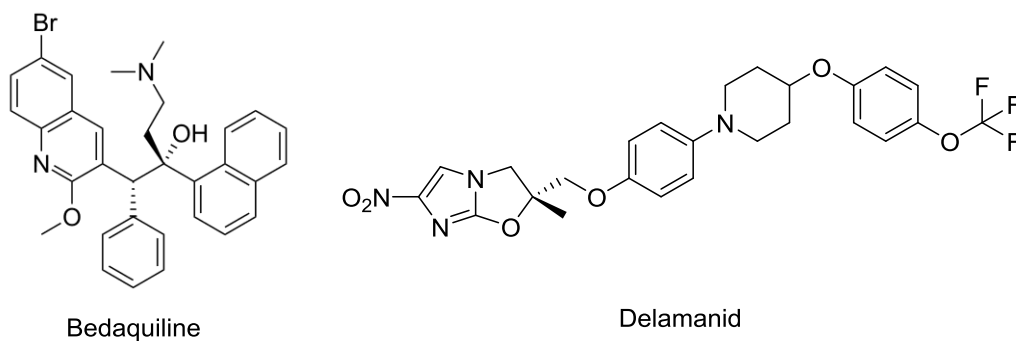


Figure 1. Current first line drugs used to treat tuberculosis and recently approved drugs for the treatment of MDR TB.

Targeting components of the Mtb respiratory chain (Figure 2) has been shown by us and other laboratories, to be effective in sterilizing both replicating and dormant Mtb.⁹⁻¹⁸ The initial target

within this programme, Ndh (Rv1854c) is a single subunit 50 KDa enzyme involved in the redox reaction of NADH oxidation with subsequent menaquinol production. Ndh has been biochemically identified as a “choke point” and as such is essential for cell function and viability.¹⁹ Essentiality of *ndh* has been shown by the inability of *Mtb* to tolerate insertion mutations in this gene²⁰ and more recently in a study involving *ndh* knockout with subsequent confirmation by complementation.²¹ The other NADH-dependent electron donating dehydrogenases identified in the genome (Complex I and *ndhA*) have been shown not to be lethal.^{18, 22} These data are consistent with biochemical evidence that Ndh is a major source of electrons for the ETC.

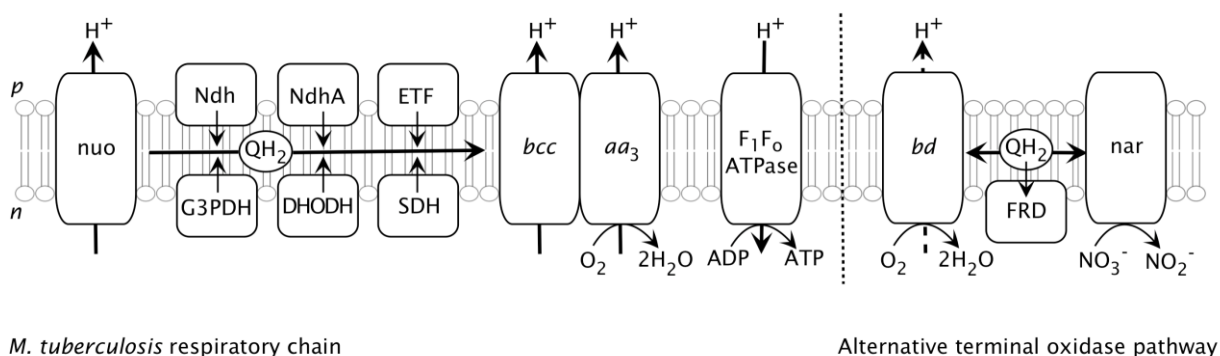


Figure 2. Schematic representation of the respiratory chain of *M. tuberculosis*. The chain components are Ndh/NdhA – type II NADH:(mena)quinone oxidoreductase (two isoforms), ETF – electron transferring flavoprotein (transfer of reducing equivalents from fatty acid β -oxidation into the Q-pool), *nuo* – protonmotive NADH dehydrogenase (Complex I), *bcc* – cytochrome *bcc* complex (note that there is no evidence for soluble cytochrome c in this organism), *aa₃* – cytochrome *bcc* oxidase, postulated to form a supercomplex with *bcc*. An alternative terminal oxidase pathway is utilised in *M. tuberculosis* under conditions of low oxygen tension, containing quinol oxidase (cytochrome *bd*), fumarate reductase (FRD) and nitrate reductase (*nar*)

components. P and n correspond to the positive and negative sides of the respiratory membrane with respect to proton translocation. Proton movements are indicative only, and do not represent H^+/e^- ratios for the respective complexes.

Respiratory-chain inhibition-induced death represents a fundamental shift from traditional anti-tubercular drug design that have until recently relied on drugs that selectively target the replication machinery of Mtb.^{9, 23-28} Anti-tubercular drugs developed to target the respiratory pathways should therefore have the potential to have sterilizing activity against current MDR and XDR Mtb strains.

Identification of hit compounds was achieved through a HTS screen of approximately 11,000 compounds that were predicted to possess activity against the Ndh enzyme. Ndh was chosen for the HTS due to the critical role as an important dehydrogenase during growth and pathogenicity^{9, 17} and due to its tractability for heterologous expression in *E.coli* and HTS²⁹. The enzyme has been observed to be sensitive to phenothiazine-based inhibitors such as trifluoperazine and thioridazine⁹. These inhibitors have been shown by a number of different laboratories to have sterilizing activity against replicating and slow growing MDR Mtb (grown anaerobically) in both *in vitro* and *in vivo* models.^{14, 30, 31} These two compounds were used as the basis to employ a range of ligand-based chemoinformatics methods³²⁻³⁵ in the rational selection of the ~11,000 compounds for the HTS campaign (selected from a commercial library of ~750,000 compounds (Biofocus DPI)).³⁶⁻⁴⁰ Selected compounds were subject to a sequential high throughput screening campaign using an *in vitro* assay against recombinant Ndh as described previously.²⁹

In addition to the HTS screen a limited selection of 50 quinolones were also screened in-house against Mtb Ndh. These compounds were selected for their structural diversity from a library of quinolones designed to target the NADH:ubiquinone oxidoreductase within the malaria parasite *Plasmodium falciparum* (PfNDH2) as described previously.⁴¹⁻⁴⁴ The HTS screen and in-house

screen in combination generated ~100 hits across 4 distinct templates, the most potent of which were also tested for whole cell replicating Mtb activity. Following analysis of the *in vitro* biological data, predicted DMPK properties and investigations into chemical tractability the quinolone template was selected as the most promising for further development.

In previous antimalarial discovery projects⁴¹⁻⁴⁸, the inhibitors based on the quinolone core displayed pharmacodynamics consistent of a privileged pharmacophore, with the ability to act on multiple electron transport chain (ETC) components. For example, quinolones with a dual mechanism of action against two respiratory enzymes, *Pf*NDH2 and cytochrome *bc₁* have recently been reported.⁴³ To exploit this phenomenon in this antitubercular discovery project, further screening and SAR investigations was switched to whole cell replicating TB activity. In order to fully establish the structure activity relationship (SAR) within the existing quinolone library with respect to whole cell Mtb activity a further library of ~350 compounds were screened against replicating Mtb. ~90 compounds were found to inhibit Mtb growth by >50% at 5 μ M. Four sub-templates were then identified as having moderate *in vitro* Mtb potency. The most promising of which only had a very limited number of examples (see Table S1 – Supporting Information) within the existing library but demonstrated significantly more potency, as such the template based on compounds **1** and **2** was chosen for lead optimisation (Figure 3).

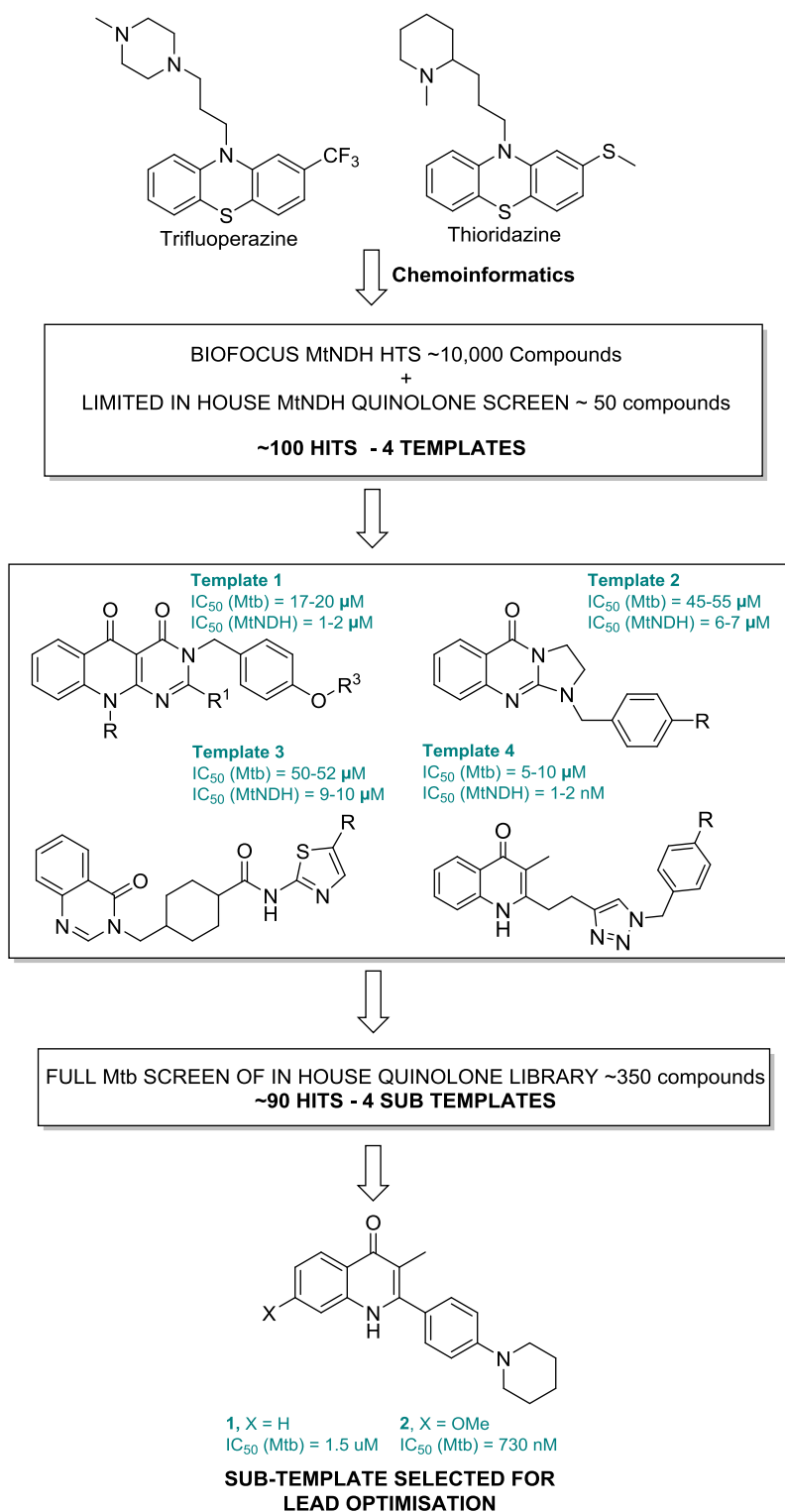


Figure 3. Identification of the quinolone template for lead optimisation.

A comprehensive medicinal chemistry SAR study around this series was then undertaken to establish optimised leads for further development. Screening data analysis (see Table S1 – Supplementary Information) shows NH₂ and OAc at the 4-position are inactive for this particular sub-template (Table S1 - entries 20, 23 and 24) and show reduced activity for other quinolone sub-templates. Replacement of the phenyl ring with a pyridyl ring also rendered the sub-template inactive (Table S1 - entry 20). Modification of ring C resulting in loss of *in vitro* Mtb potency is a general trend that was seen across most quinolone sub-templates screened. Modifications of particular interest were therefore optimization of the side chain to optimize potency and DMPK, the nature of the group at 3-position and the electronic/steric effect of substituents placed at the 5, 6 and 7 positions (Figure 4).

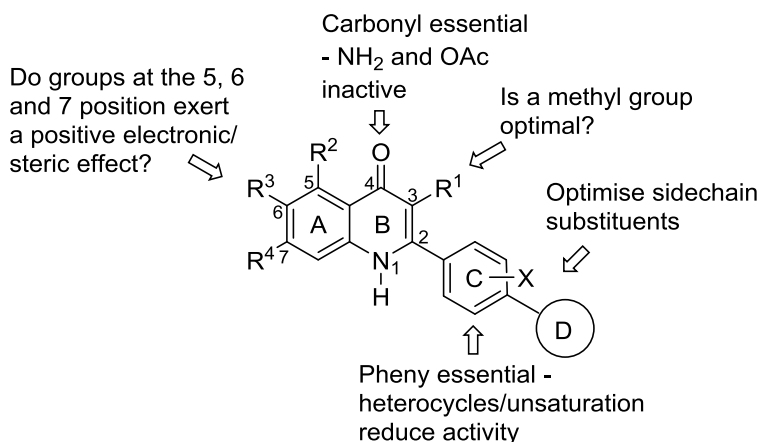


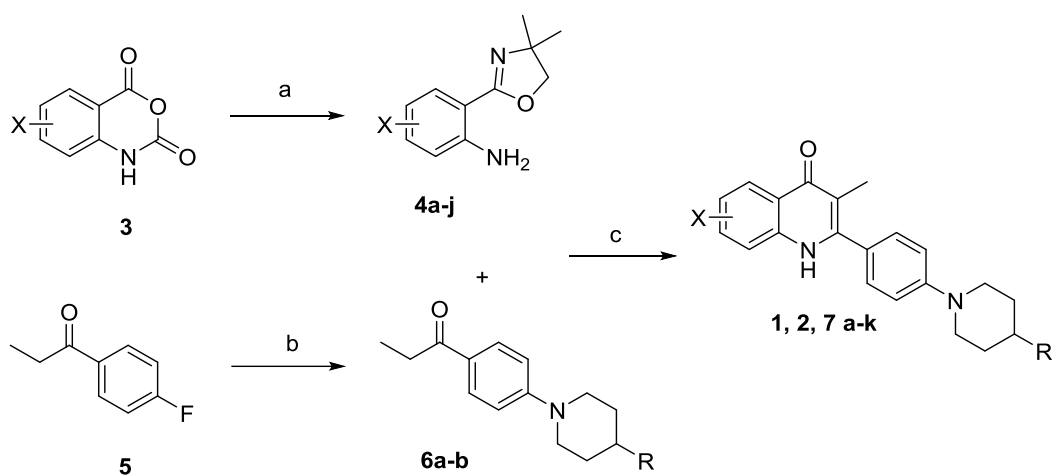
Figure 4. Known SAR and SAR to be investigated.

CHEMISTRY

Following identification of quinolones **1** and **2** as the initial hits against Mtb, our initial efforts were focused on exploring the SAR of substituents placed in the A ring. The synthesis of these compounds was achieved in 3 – 5 steps from commercially available starting materials (Scheme

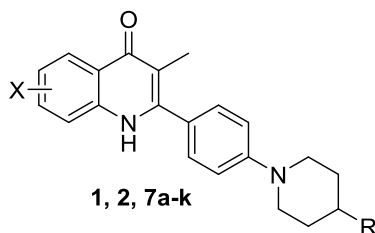
1). Oxazoline **4** was prepared from the corresponding isatoic anhydride **3** in yields of 34 – 75%. Where the isatoic anhydride was not commercially available, the oxazolines were synthesized in-house (see Supporting information). 4'-fluoropropiophenone **5** was reacted with piperidine to give ketone **6** in 32 – 97% yields. Reaction of oxazoline **4** with ketone **6** in the presence of triflic acid gave the desired quinolones **1**, **2**, **7a-k** in 23 – 45% yields.

Scheme 1. Synthesis of Quinolones **1**, **2**, **7a-k**.^a



^a Conditions and reagents: (a) 2-amino-2-methyl-propanol, ZnCl_2 , PhCl , 135 °C, 24 h; (b) corresponding amine, K_2CO_3 , DMF, 120°C to reflux, overnight ; (c) $\text{CF}_3\text{SO}_3\text{H}$, *n*-BuOH, N_2 , 130 °C, 24 h.

Table 1. Yields for the Synthesis of Compounds **1**, **2**, **7a-k**.

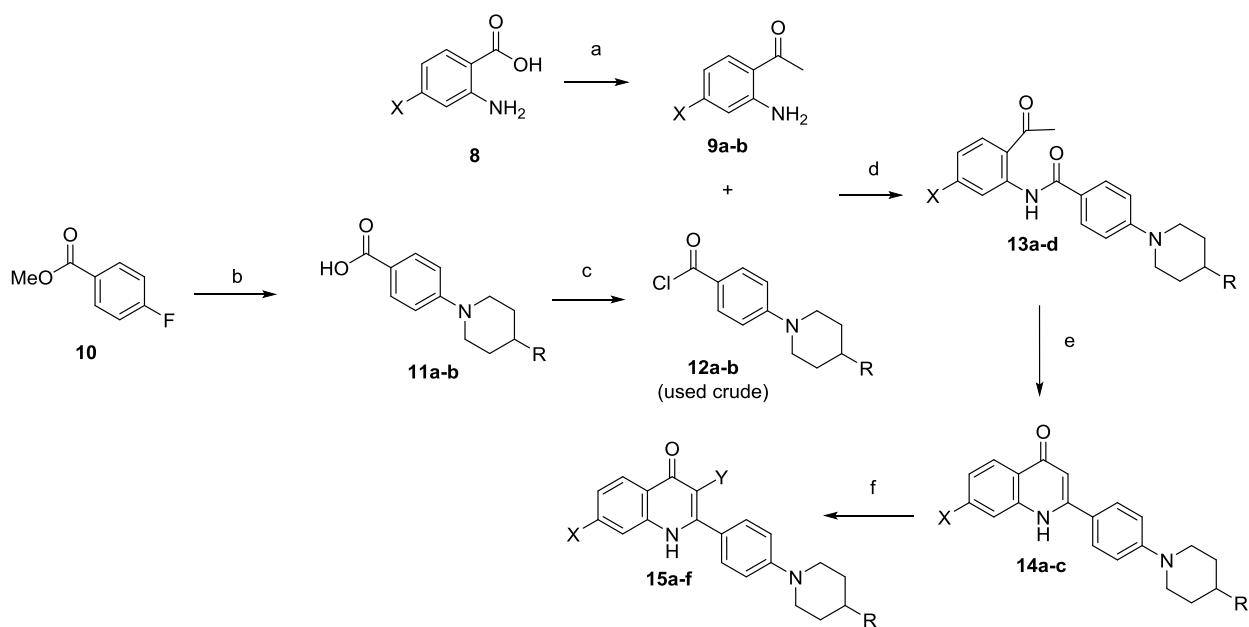


| Compound | X | R | % yield 4 | % yield 6 | % yield 7 |
|-----------|--------------|---|------------------|------------------|------------------|
| 1 | H | H | - | 62 | 23 |
| 2 | 7-OMe | H | 75 | 62 | 36 |
| 7a | 6-F | H | 60 | 62 | 26 |
| 7b | 6-OMe, 7-OMe | H | 52 | 62 | 28 |
| 7c | 6-Cl, 7-OMe | H | 45 | 62 | 35 |
| 7d | 6-F, 7-OMe | H | 52 | 62 | 41 |
| 7e | 5-OMe, 7-OMe | H | 58 | 62 | 32 |
| 7f | 5-F,7-F | H | 68 | 62 | 45 |
| 7g | 7-F | H | - | 62 | 35 |
| 7h | 7-Cl | H | 64 | 62 | 37 |
| 7i | H | F | - | 55 | 36 |
| 7j | 7-OMe | F | 75 | 55 | 43 |
| 7k | 5-F,7-F | F | 68 | 55 | 29 |

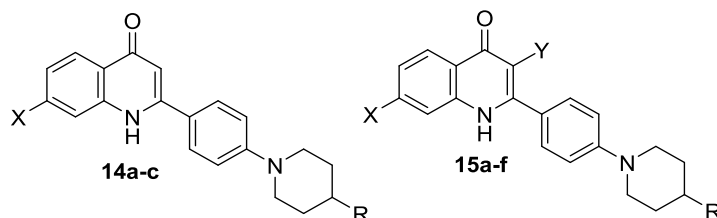
The nature of the group at 3-position of the quinolone was also studied. A small set of analogues with a hydrogen at 3-position were synthesized (Scheme 2). Substituted 2-aminoacetophenone **9** was converted from the respective aminobenzoic acid **8** using methyl lithium in 36% yield. 4-fluorobenzoate **10** was reacted with piperidine in the presence of potassium carbonate to give the piperidinyl benzoate **11** in 37% yield. Benzoate **11** was hydrolysed to benzoic acid which was then converted to acid chloride **12** by oxalyl chloride. Acylation of 2-aminoacetophenone **9** with acid chloride **12** provided the intermediate **13** in 30 – 51% yields. Cyclisation of the intermediate **13** in the presence of NaOH or KO^tBu gave the 3-H quinolones **14a-c** in 41 – 91% yields (Table 2).

Literature precedent from the development of ETC inhibitors in the antimalarial field lead us then to look at the presence of a halide at the 3-position. GSK's pyridone series⁴⁹ demonstrated tolerance of the presence of a chlorine at 3-position and within our own group we have shown the combination of 3-chloro-7-methoxy enhances biological activity of the quinolone core.⁵⁰ In order to achieve this the 3-H compounds were treated with sodium dichloroisocyanurate and sodium hydroxide to give 3-Cl quinolones **15a-d** in 40 – 61% yields, or NBS to give 3-Br quinolones **15e-f** in 55 – 63% yields.

Scheme 2. Synthesis of quinolones **14 a-c** and **15 a-f**.^a



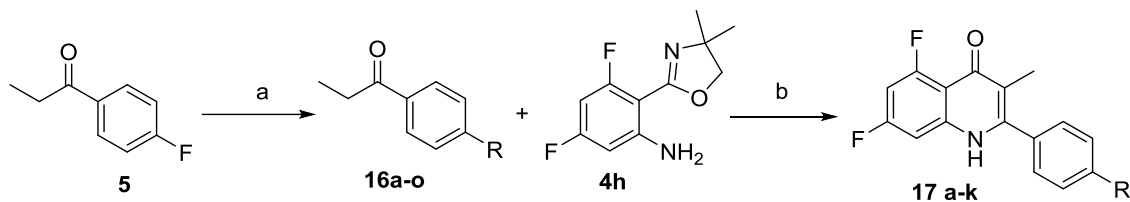
^a Conditions and reagents: (a) MeLi, DME, 0°C, 2 h; (b) (i) K₂CO₃, DMF, reflux, overnight, (ii) NaOH (aq), MeOH, reflux, overnight; (c) oxalyl chloride, DCM, DMF (cat.), r.t., 2 h; (d) NEt₃, THF, r.t., overnight; (e) NaOH (s), 1,4-dioxane, 110°C, 5 h or KO^tBu, ^tBuOH, 75°C, 16 h; (f) sodium dichloroisocyanurate, 1M NaOH (aq), MeOH, r.t., overnight (**15a-d**) or NBS, DCM, DMF, r.t., overnight (**15e-f**).

Table 2. Yields for the Synthesis of Compounds **14a-c** and **15a-f**.

| Compound | X | R | Y | % yield 9 | % yield 11 | % yield 13 | % yield 14 | % yield 15 |
|------------|-----|---|----|---------------------|----------------------|----------------------|----------------------|----------------------|
| 14a | H | H | H | - | - | 51 | 70 | - |
| 14b | OMe | H | H | 36 | - | 50 | 41 | - |
| 14c | OMe | F | H | 36 | 37 | 30 | 68 | - |
| 15a | H | H | Cl | - | - | 51 | 70 | 40 |
| 15b | OMe | H | Cl | 36 | - | 50 | 41 | 61 |
| 15c | OMe | F | Cl | 36 | 37 | 30 | 68 | 52 |
| 15d | H | F | Cl | - | 37 | 60 | 91 | 58 |
| 15e | H | H | Br | - | - | 51 | 70 | 63 |
| 15f | H | F | Br | - | 37 | 60 | 91 | 55 |

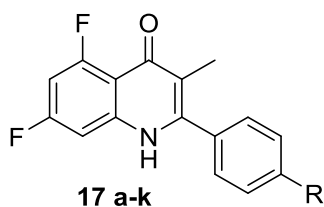
Having identified 3-methyl and 5, 7-difluoro quinolone (followed by 6-fluoro-7-methoxy and 7-methoxy quinolone) to be optimal for Mtb activity (see Table 8), the focus of SAR explorations moved to the terminal ring of the side chain to further improve Mtb activity and optimise DMPK. Additional small groups, such as Me, F and CF₃ attached at different positions on the terminal piperidine ring were investigated. In addition the effect of chirality was explored.⁵¹ Synthesis of compounds **17a-k** was achieved using chemistry described in Scheme 3.

Scheme 3. Synthesis of Quinolones **17a-k**.^a



^a Conditions and reagents: (a) corresponding amine, K₂CO₃, DMF, 120°C to reflux, overnight ; (b) CF₃SO₃H, *n*-BuOH, N₂, 130 °C, 24 h.

Table 3. Yields for the Synthesis of Compounds **17a-k**.

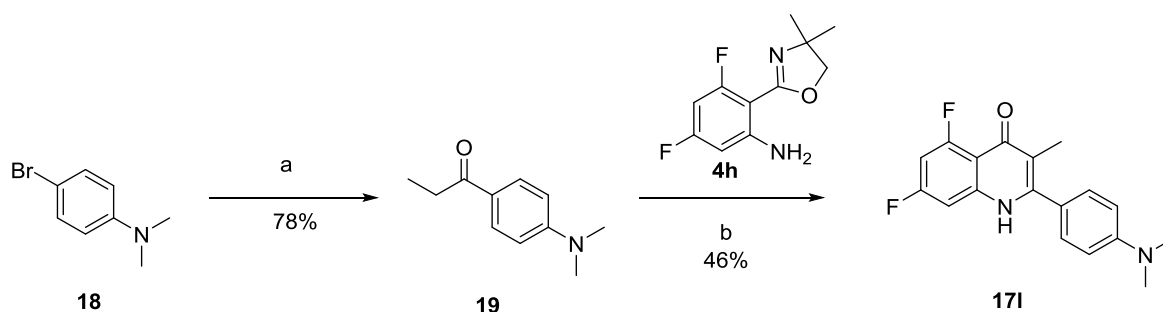


| Compound | R | % Yield 16 | % Yield 17 |
|------------|---|-------------------|-------------------|
| 17a | | 48 | 32 |
| 17b | | 73 | 54 |
| 17c | | 48 | 34 |
| 17d | | 40 | 57 |
| 17e | | 64 | 27 |
| 17f | | 84 | 45 |
| 17g | | 74 | 43 |
| 17h | | 75 | 40 |
| 17i | | 72 | 39 |

| | | | |
|------------|---|----|----|
| 17j |  | 69 | 41 |
| 17k | -NHCH ₂ Ph | 28 | 51 |

Incorporation of different amino groups into the side chain as an alternative to the potentially metabolically labile piperidine ring was also investigated. To incorporate a diethylamine group an alternative methodology was used to synthesise the side chain, commercial available 4-bromo-*N,N*-dimethylaniline **18** was treated with butyllithium for a lithium-halogen exchange and the intermediate was quenched with *N,N*-dimethylpropionamide to form the side chain **19** in 78% yield, reaction with oxazoline **4h** was then carried out to give quinolone **17l** in 46% yield (Scheme 4).

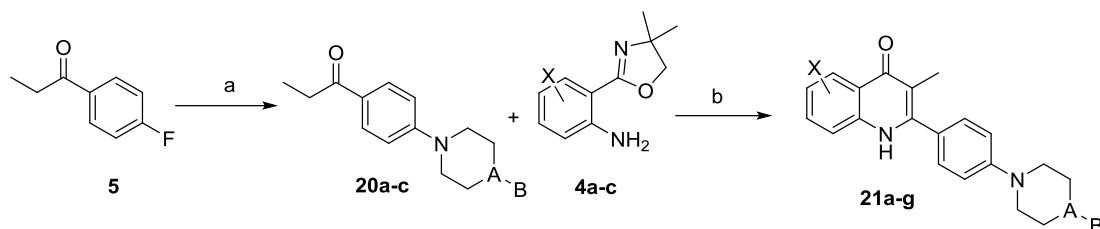
Scheme 4. Synthesis of quinolone **17l**.^a



^a Conditions and reagents: (a) (i) *n*BuLi, Et₂O, -78°C, 30 min; (ii) *N,N*-dimethylpropionamide, -78°C to r.t., 2 h; (b) CF₃SO₃H, *n*-BuOH, N₂, 130 °C, 24 h.

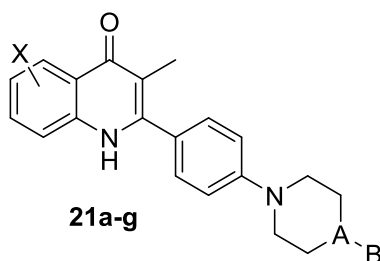
Extension of the side chain with a phenyl or benzyl group at the 2-position was also investigated using the synthetic methodologies shown in Scheme 5. In addition, replacement of piperidine by piperazine was investigated. This was to further explore the length of side chain that could be tolerated and to improve the solubility.

Scheme 5. Synthesis of Quinolones **21a-g**.^a



^a Conditions and reagents: (a) corresponding amine, K₂CO₃, DMF, 120°C to reflux, overnight; (b) CF₃SO₃H, *n*-BuOH, N₂, 130 °C, 24 h.

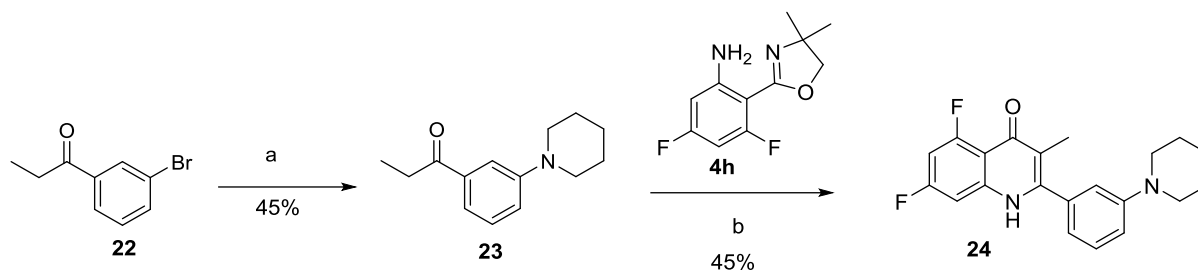
Table 4. Yields for the Synthesis of Compounds **21a-g**.



| Compound | X | A | B | % Yield 20 | % Yield 21 |
|------------|-------|----|--------------------|-------------------|-------------------|
| 21a | H | CH | CH ₂ Ph | 64 | 33 |
| 21b | 6-F | CH | CH ₂ Ph | 64 | 40 |
| 21c | 7-OMe | CH | CH ₂ Ph | 64 | 42 |
| 21d | H | N | CH ₂ Ph | 58 | 30 |
| 21e | 6-F | N | CH ₂ Ph | 58 | 28 |
| 21f | 7-OMe | N | Ph | 64 | 30 |
| 21g | 7-OMe | N | CH ₂ Ph | 58 | 38 |

In addition, the quinolone with a piperidine ring at the meta-position **24** was also synthesised by reacting the 3-bromopropiophenone **22** with piperidine using Buchwald coupling to yield the ketone intermediate **23**, which was coupled with oxazoline **4h** to give the quinolone in 45% yield (Scheme 6).



Scheme 6. Synthesis of Quinolone **24**.^a



^a Conditions and reagents: (a) Piperidine, Pd(OAc)₂, XPhos, NaO^tBu, Toluene, 110 °C, 24 h; (b) CF₃SO₃H, *n*-BuOH, N₂, 130 °C, 24 h.

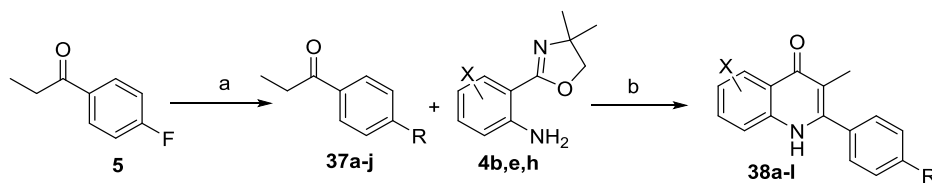
A series of analogues with a pyrrole heterocycle in the side chain were also synthesized to further explore the side chain SAR and enhance the metabolic stability. The synthetic route to these compounds is illustrated in Scheme 7. Utilizing Copper and trans-*N,N'*-Dimethyl-1,2cyclohexanediamine catalyzed *N*-arylation with 4-bromopropiophenone the side chain ketone intermediate **31** was formed in 30 – 62 % yields.^{52, 53} Final cyclisation with oxazoline gave quinolones **32a-g** in 35 – 57% yields.

Scheme 7. Synthesis of quinolones **32a-g**.^a

| | | | | | |
|------------|---------|--------------|---|----|----|
| 32f | 5-F,7-F | <i>m</i> -Cl |  | 49 | 39 |
| 32g | 5-F,7-F | <i>o</i> -F |  | 52 | 39 |

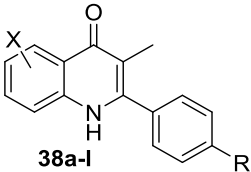
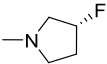
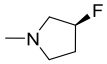
Using fluorine to block metabolism and improve oral absorptions was further explored. Research by Smith has shown that gem-difluorinated piperidine compounds exhibited a significant improvement in metabolic stability.⁵⁴ This led to the design and synthesis of fluorinated quinolones **38a-f** as well as the alcohol side chain quinolones **38g-i**. The chemistry used in the synthesis of these compounds is shown in Scheme 8.

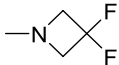
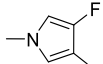
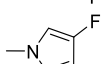
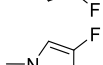
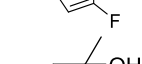
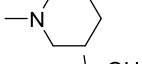
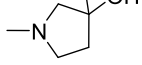
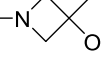
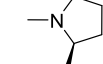
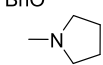
Scheme 8. Synthesis of Quinolones **38a-l**.^a



^a Conditions and reagents: (a) corresponding amine, K₂CO₃, DMF, 120°C to reflux, overnight; (b) CF₃SO₃H, *n*-BuOH, N₂, 130 °C, 24 h.

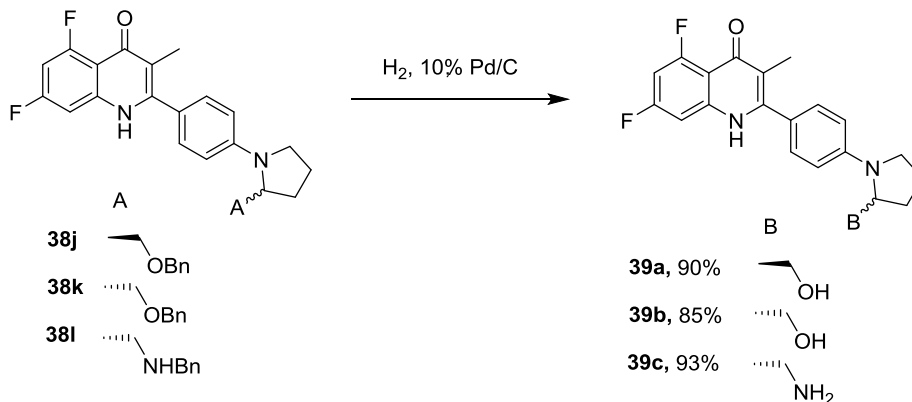
Table 6. Yields for the Synthesis of Compounds **38a-l**.

|  | | | | |
|---|---------|---|-------------------|-------------------|
| Compound | X | R | % Yield 37 | % Yield 38 |
| 38a | 5-F,7-F |  | 38 | 45 |
| 38b | 5-F,7-F |  | 37 | 47 |

| | | | | |
|------------|------------|---|----|----|
| 38c | 5-F,7-F |  | 25 | 33 |
| 38d | 5-F,7-F |  | 32 | 30 |
| 38e | 7-OMe |  | 32 | 32 |
| 38f | 6-Cl,7-OMe |  | 32 | 30 |
| 38g | 5-F,7-F |  | 69 | 48 |
| 38h | 5-F,7-F |  | 54 | 50 |
| 38i | 5-F,7-F |  | 32 | 43 |
| 38j | 5-F,7-F |  | 41 | 20 |
| 38k | 5-F,7-F |  | 43 | 25 |
| 38l | 5-F,7-F |  | 41 | 37 |

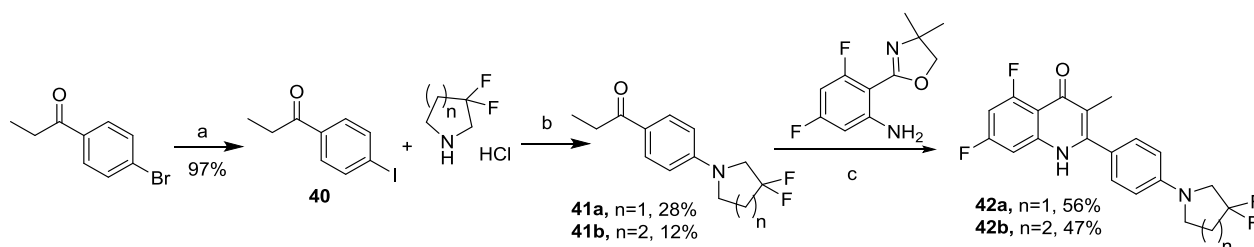
Removal of the benzyl group from the chiral proline derivatives **38j-l** was achieved using hydrogenation (Scheme 9) in good yields.

Scheme 9. Synthesis of compounds **39a-c**.



For the gem-difluoro analogues (**42a** (MTC420) and **42b**), 4-bromopropiophenone was first converted to a more reactive 4-iodopropiophenone by an aromatic Finkelstein reaction catalysed by copper(I) iodide in combination with *N,N*-dimethyl-1,2-diaminoethane.⁵⁵ A subsequent Buchwald-Hartwig amination using Pd₂(dba)₃ and Xantphos with the gem-fluorinated amine gave the ketone side chain **41a-b** in 12 – 28% yields.⁵⁶ Reaction with oxazoline gave quinolones **42a-b** in 47 – 56% yields (Scheme 10).

Scheme 10. Synthesis of quinolones **42a-b**.^a

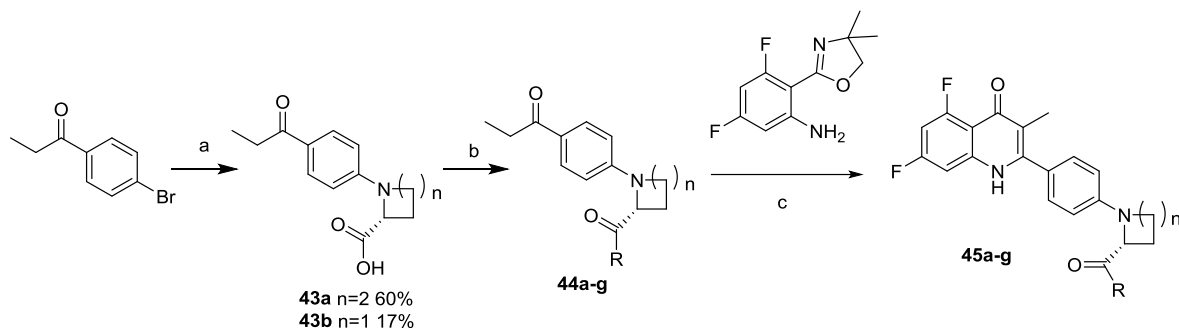


^a Conditions and reagents: (a) CuI, *N,N*-dimethyl-1,2-diaminoethane, NaI, 1,4-dioxane, 110°C, 24 h; (b) Pd₂(dba)₃, Xantphos, NaO^tBu, 1,4-dioxane, 110°C, 24 h; (c) CF₃SO₃H, *n*-BuOH, N₂, 130 °C, 24 h.

42a was identified as the lead compound in the series as it exhibited good potency and metabolic stability (See Table 11 and Table 12), further investigation of the pyrrolidine side chain was undertaken to improve solubility and potency. Further modifications have included adding chirality and introducing amide functionality to rapidly ascertain if it is tolerated within the template. Quinolones **45a-h** were therefore synthesised using chemistry described in Scheme 11. To incorporate the amide group, Ullmann coupling of 4-bromopropiophenone with *D*-proline gave the carboxylic acid intermediate **43a-b**. Crosslinking the carboxylic acid by EDC/NHS to

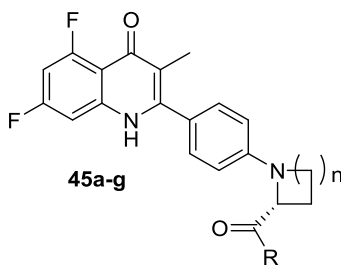
respective amine provided the ketone side chain **44** in 52 – 90% yields. This was subsequently coupled with oxazoline in 12 – 34% yields to afford quinolones **45a-g**.

Scheme 11. Synthesis of Quinolones **45a-g**.^a



^a Conditions and reagents: (a) D-proline, CuI, K₂CO₃, DMF, 140°C, 24 h; (b) (i) EDC, N-hydroxysuccinamide, CHCl₃, NEt₃, amine, r.t., 6 h; (ii) amine, NEt₃, r.t., 2h; (c) CF₃SO₃H, *n*-BuOH, N₂, 130 °C, 24 h.

Table 7. Yields for the Synthesis of Compounds **45a-g**.

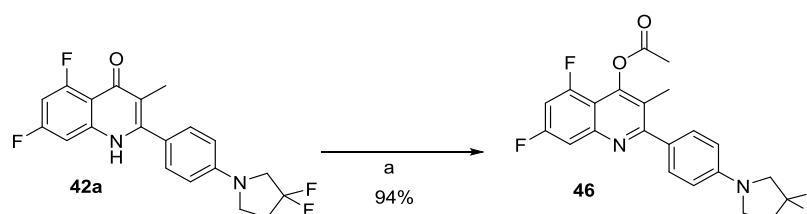


| Compound | R | n | % yield 44 | % yield 45 |
|------------|---------------------|---|-------------------|-------------------|
| 45a | -NH ^t Bu | 2 | 52 | 20 |
| 45b | -NEt ₂ | 2 | 80 | 34 |
| 45c | | 2 | 90 | 25 |
| 45d | | 2 | 70 | 18 |
| 45e | | 2 | 65 | 24 |
| 45f | -NMe ₂ | 1 | 45 ^a | 15 |

| | | | | |
|---|---------------------|---|----------------|----|
| 45g | -NH ^t Bu | 1 | - ^b | 12 |
| ^a Alternative methodology used please see supporting information. ^b Used crude. | | | | |

Incorporation of an amide moiety largely resulted in reduced anti-tuberculosis activity (Table 7). As such our attention returned to **42a** and improving its pharmacokinetic profile. Use of a pro-drug strategy, previously used successfully within other quinolone development programs⁵⁷ was investigated leading to the synthesis of compound **46**. Compound **46** was synthesized by reacting **42a** with potassium *tert*-butoxide and acetyl chloride to give the acetate pro-drug in high yield.

Scheme 12. Synthesis of pro-drug **46**. ^a



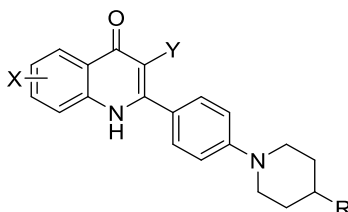
^a (a) i. ^tBuOK, THF, r.t., 1h. ii. Acetyl chloride, r.t., 3h.

RESULTS AND DISCUSSION

Structure Activity Relationships (SAR) - Initial SAR investigations around the hit compounds **1** and **2** focused on establishing the optimal A-ring substituents (X). Compounds **1**, **2** and **7a-7h** demonstrate the most favorable X groups are 5-F, 7-F closely followed by 6-F, 7-OMe and 7-OMe. Compounds **7i-7k** were synthesised with a view to reducing the potential metabolism of the piperidine ring. Pleasingly a good level of potency was maintained. Concomitantly the potential for replacing the methyl group at Y was also investigated. When Y=H activity is lost as demonstrated by compounds **14a-c**. Halogenation was also investigated; again this largely resulted in reduced anti-tuberculosis activity (**15a-f**). The one exception to this being **15e** possessing a Br

at Y. This affect appeared to be compound specific rather than a general trend across all brominated analogues and as such it was decided that the methyl group was the optimal group at this position.

Table 8. Mtb IC₅₀ values for compounds **1**, **2**, **7a-k**, **14a-c** and **15a-f**.



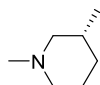
| Compound | X | Y | R | Mtb IC ₅₀ (μM) |
|------------|--------------|----|---|---------------------------|
| 1 | H | Me | H | 1.50 ± 0.19 |
| 2 | 7-OMe | Me | H | 0.73 ± 0.01 |
| 7a | 6-F | Me | H | 1.83 ± 0.22 |
| 7b | 6-OMe, 7-OMe | Me | H | >10 |
| 7c | 6-Cl, 7-OMe | Me | H | >10 |
| 7d | 6-F, 7-OMe | Me | H | 0.52 ± 0.06 |
| 7e | 5-OMe, 7-OMe | Me | H | >10 |
| 7f | 5-F, 7-F | Me | H | 0.27 ± 0.08 |
| 7g | 7-F | Me | H | >10 |
| 7h | 7-Cl | Me | H | >10 |
| 7i | H | Me | F | >10 |
| 7j | 7-OMe | Me | F | 1.32 ± 0.10 |
| 7k | 5-F, 7-F | Me | F | 0.94 ± 0.12 |
| 14a | H | H | H | >10 |
| 14b | 7-OMe | H | H | >10 |
| 14c | 7-OMe | H | F | >10 |
| 15a | H | Cl | H | 1.56 ± 0.22 |
| 15b | 7-OMe | Cl | H | 2.82 ± 0.21 |
| 15c | 7-OMe | Cl | F | >10 |
| 15d | H | Cl | F | >10 |
| 15e | H | Br | H | 0.60 ± 0.09 |
| 15f | H | Br | F | >10 |

With 5-F, 7-F and 3-methyl confirmed as optimal for anti-tuberculosis activity optimising the side chain then became the focus of the SAR studies (Table 9). Initial investigations into piperidine ring substituents at the 4-position revealed that in addition to 4-F **7k**, a methyl group is also

tolerated as demonstrated with compound **17b**. It rapidly became apparent that there was a size limitation to the group tolerated at the 4-position with larger groups such as CF₃, cyclopropyl and gem-difluoro resulting in loss of potency. Movement of the F and Me groups to the 3-position resulted in improvements in anti-tuberculosis activity as demonstrated by compounds **17e-h**. Interestingly racemic and enantiomerically pure analogues of the 3-methyl derivative **17f** showed little variation in potency, which is in direct contrast to the pyrrolidine analogues discussed later. Replacement of the piperidine ring with a number of alternative amines was also investigated. Increasing ring size (**17j**) and use of dimethyl amine (**17l**) retained good potency. Incorporation of secondary amines (**17k**) and more polar groups such as *N*-methyl piperazine (**17i**) reduced anti-tuberculosis activity. Moving the piperidine group from the *para* to the *meta*-position (**24**) also resulted in loss of activity.

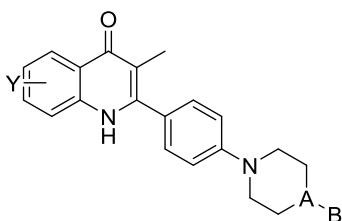
Table 9. Mtb IC₅₀ values for compounds **17a-l** and **24**.

| Compound | R | Mtb IC ₅₀ (μM) | Compound | R | Mtb IC ₅₀ (μM) |
|------------|---|---------------------------|------------|-----------------------|---------------------------|
| 17a | | >10 | 17h | | 0.47 ± 0.02 |
| 17b | | 0.61 ± 0.05 | 17i | | >10 |
| 17c | | >10 | 17j | | 0.49 ± 0.07 |
| 17d | | >10 | 17k | -NHCH ₂ Ph | >10 |
| 17e | | 0.31 ± 0.03 | 17l | | 0.41 ± 0.002 |
| 17f | | 0.37 ± 0.04 | 24 | | >10 |

17g 0.47 ± 0.03

The size limitation and unfavorable incorporation of piperazine was further confirmed by our concomitant investigation in to extended side chain analogues (Table 10). The aim of this series was to explore the space available and to improve solubility with the incorporation of piperazine to facilitate salt based formulation.

Table 10. Mtb IC₅₀ values for compounds **21a-g**.



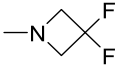
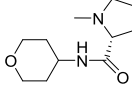

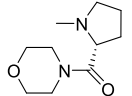

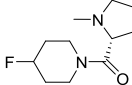

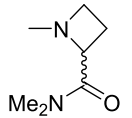
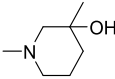
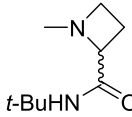
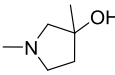
| Compound | X | A | B | Mtb IC ₅₀ (μM) |
|------------|-------|----|--------------------|---------------------------|
| 21a | H | CH | CH ₂ Ph | >10 |
| 21b | 6-F | CH | CH ₂ Ph | >10 |
| 21c | 7-OMe | CH | CH ₂ Ph | >10 |
| 21d | H | N | CH ₂ Ph | 5.74 ± 0.66 |
| 21e | 6-F | N | CH ₂ Ph | >10 |
| 21f | 7-OMe | N | Ph | >10 |
| 21g | 7-OMe | N | CH ₂ Ph | >10 |

With this information in hand several small heterocyclic, fluorinated, chiral and amide analogues were synthesized to investigate SAR and improve DMPK (Table 11). Compounds **32a-g** are pyrrole derivatives. An unsubstituted pyrrole moiety is well tolerated in the 5-F (**32d**) and 5-F, 7-F (**32e**) analogues, however increasing the size of the pyrrole group by addition of a fused benzene

ring (**32b**) again results in loss of potency. Incorporation of a halogen on the aromatic ring was also investigated but reduced potency.

Table 11. Mtb IC₅₀ values for compounds **32a-g**, **38a-j**, **39a-c**, **42a-b** and **45a-g**.

| Compound | X | R | Mtb IC ₅₀ (μM) | Compound | X | R | Mtb IC ₅₀ (μM) |
|------------|-----------------------------|---|---------------------------|------------|---------|---|---------------------------|
| 32a | 5-F,7-F | | >10 | 38i | 5-F,7-F | | >10 |
| 32b | 5-F,7-F | | >10 | 38j | 5-F,7-F | | 0.96 ± 0.06 |
| 32c | 5-F,7-F | | >10 | 39a | 5-F,7-F | | 1.59 ± 0.05 |
| 32d | 5-F | | 0.71 ± 0.05 | 39b | 5-F,7-F | | 0.32 ± 0.04 |
| 32e | 5-F,7-F | | 0.44 ± 0.02 | 39c | 5-F,7-F | | >10 |
| 32f | 5-F,7-F Y = <i>m</i> -Cl | | >10 | 42a | 5-F,7-F | | 0.53 ± 0.08 |
| 32g | 5-F,7-F Y = <i>o</i> -F | | >10 | 42b | 5-F,7-F | | 0.36 ± 0.04 |
| 38a | 5-F,7-F | | 0.23 ± 0.003 | 45a | 5-F,7-F | | 0.96 ± 0.05 |
| 38b | 5-F,7-F | | 1.80 ± 0.09 | 45b | 5-F,7-F | | >10 |

| | | | | | | | |
|------------|------------|---|-------------|------------|---------|---|-----|
| 38c | 5-F,7-F |  | 1.53 ± 0.04 | 45c | 5-F,7-F |  | >10 |
| 38d | 5-F,7-F |  | >10 | 45d | 5-F,7-F |  | >10 |
| 38e | 7-OMe |  | >10 | 45e | 5-F,7-F |  | >10 |
| 38f | 6-Cl,7-OMe |  | >10 | 45f | 5-F,7-F |  | >10 |
| 38g | 5-F,7-F |  | 5.01 ± 0.03 | 45g | 5-F,7-F |  | >10 |
| 38h | 5-F,7-F |  | >10 | | | | |

Fluorinated analogues were synthesized in order to improve metabolic stability (see Table 11). Both mono (**38a** and **38b**) and gem-difluoro (**42a**) substituted pyrrolidine derivatives exhibited good to excellent potency. The gem-difluoro azetidine (**38c**) and 3-substituted piperidine (**42b**) also demonstrated good potency. Incorporation of an alcohol group in the side chain to reduce lipophilicity and potentially facilitate pro-drug approaches provided mixed results. Gem-methyl, OH analogues **38g-i** were not tolerated whereas inclusion of prolinol (**39a-b**) gave good anti-tuberculosis activity. Benzylated analogue **38j** and amide analogues **45a-g** largely resulted in loss of potency. For the pyrrolidine analogues the effect of chirality on activity was marked with the (*R*)-3-fluoro analogue **38a** (Mtb IC₅₀ = 0.23 μM) demonstrating significantly superior potency over the (*S*)-3-fluoro analogue **38b** (Mtb IC₅₀ = 1.80 μM). The effect of chirality was also observed with the prolinol analogues, (*S*)-prolinol analogue **39b** (Mtb IC₅₀ = 0.32 μM) being more active

than (*R*)-prolinol analogue **39a** (Mtb IC₅₀ = 1.52 μM). The overall SAR trends for the series can be seen in Figure 5.

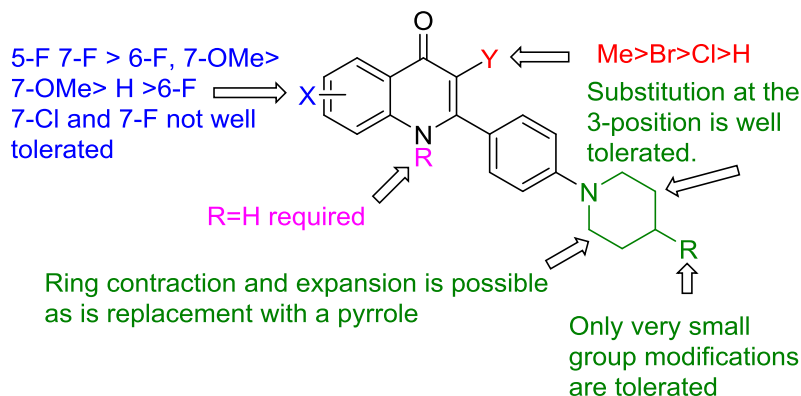


Figure 5. Overall SAR trends for the heterocyclic quinolone series.

***In vitro* DMPK and toxicity** - Analogues demonstrating good potency were then moved through our screening cascade and evaluated for microsomal turnover and HEPG2 cytotoxicity. None of the compounds were found to be cytotoxic and all had good therapeutic indexes. From the earlier analogues tested (entries 1-6 in Table 12) it was apparent that the compounds were being metabolised quickly by liver microsomes. Resolving this issue was therefore the driving force for a large proportion of the medicinal chemistry manipulations described in Table 11 above.

Table 12. HEPG2 and microsomal turnover t_{1/2} for selected analogues.

| Compound | Mtb IC ₅₀ (μM) | Mtb IC ₉₀ (μM) | HEPG2 GLU (μM) | Therapeutic Index | Microsomal Turnover (h, m, r) t _{1/2} (min) |
|-----------|---------------------------|---------------------------|----------------|-------------------|--|
| 7f | 0.270 ± 0.080 | 0.78 | >100 | >370 | h-7.31 m-8.27 r-8.30 |
| 7k | 0.950 ± 0.120 | 1.83 | 102.2 | 108 | h-5.7 m-4.4 r-8.4 |

| | | | | | |
|------------|---------------|------|-------|------|-----------------------------|
| 17b | 0.611 ± 0.048 | 1.93 | >100 | >164 | h-<10 m-<10 r-<10 |
| 17e | 0.300 ± 0.025 | 0.56 | 188.1 | 627 | h-7.8 m-6.8 r-10.1 |
| 17f | 0.367 ± 0.040 | 0.63 | >100 | >272 | h-7.9 m-22.3 r-10.8 |
| 17h | 0.400 ± 0.023 | 0.66 | 85.54 | 223 | h-8.54 m-7.65 r- 5.72 |
| 32e | 0.432 ± 0.020 | 0.69 | 141 | 342 | h-10.2 m-20.7 r-30.1 |
| 38a | 0.231 ± 0.036 | 0.50 | 150.6 | 649 | h-10.2 m-4.4 r-10.6 |
| 38d | >10 | >10 | ND | ND | h-60 m-60 r-60 |
| 42a | 0.525 ± 0.080 | 1.10 | >100 | >190 | h-72.8 m-114.9 r-61.6 |
| 42b | 0.361 ± 0.041 | 0.83 | ND | ND | h-17.4 m-16.2 r-13.7 |

Two strategies were employed to address the metabolic stability issues (Figure 6). The first was to replace the piperidine ring with an alternative heterocycle. Amongst those selected pyrrole (**32e**) provided the most active compound with a modest improvement in metabolic stability. Fluorination of the pyrrole (**38d**) at the 3 and 4 positions resulted in complete resolution of metabolic instability; however anti-tuberculosis activity was also lost. From earlier SAR studies we knew that replacing the piperidine ring (**7f**) with a pyrrolidine ring (**17i**) was tolerated in terms of activity and may provide us with more opportunity to modify the ring in what we believe to be a limited space. Mono-fluorination (**38a**) provided a very modest improvement in stability.

Subsequent synthesis of the gem-difluoro analogue (**42a**) however provided us with a compound with both good anti-tuberculosis activity and excellent metabolic stability. The equivalent six membered ring analogue **42b** had good potency but comparatively decreased metabolic stability as expected (Table 12).

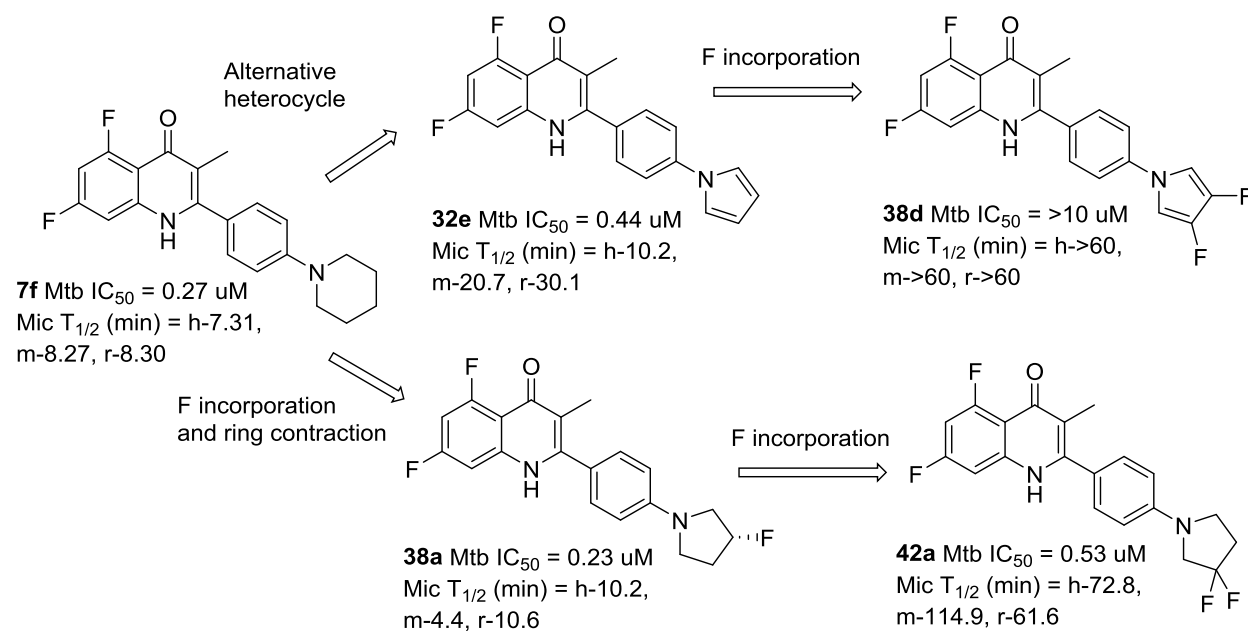


Figure 6. Resolution of metabolic stability problems.

Selected analogues were also measured for Caco-2 permeability, stability in plasma, % plasma protein binding (PPB) and solubility (Table 13). All compounds performed well in these assays with the exception of solubility which is a common issue for the quinolone chemotype.

Table 13. Caco-2 permeability, stability in plasma, % PPB and solubility values for selected analogues.

| Compound | Caco-2 permeability (cm ² /s) | Stability in plasma (r,h) T _{1/2} (min) | Human PPB (%) | Solubility (μ g/mL) | | |
|----------|---|---|---------------|--------------------------|-------|-----------------|
| | | | | pH1 | pH7.4 | CM ^a |

| | | | | | | |
|------------|--------------------------|------------------|-------|------|-----|-----|
| 5k | ND | r->180 h->180 | 95.82 | >150 | <1 | 12 |
| 17e | 22.86 x 10 ⁻⁶ | r->180 h->180 | 98.45 | >150 | <1 | 10 |
| 32e | 30.97 x 10 ⁻⁶ | r->180 h->180 | 96.1 | 5.1 | 3.6 | 61 |
| 38b | 15.51 x 10 ⁻⁶ | r->180 h->180 | 98.97 | <1 | <1 | 2.5 |
| 42a | 10.00 x 10 ⁻⁶ | r->180 h->180 | 97.30 | < 1 | < 1 | 55 |

^a CM – culture media - Middlebrook 7H9 broth with addition of 10% albumin–dextrose–catalase solution (Becton Dickinson), 0.2% [vol/vol] glycerol and 0.05% [vol/vol] Tween 80.

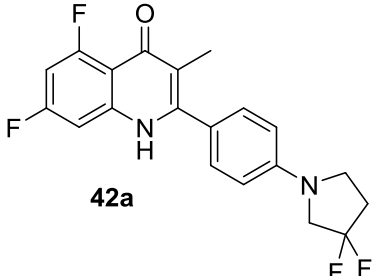
A number of analogues also underwent additional *in vitro* DMPK (Table 14) experiments further confirming the metabolism issues detailed above.

Table 14. *In vitro* DMPK measurements for selected analogues.

| Compound | Aqueous Solubility (μM) | Human % PPB | LogD7.4 | Human Microsomes CLint (μL/min/mg) | Rat Hepatocytes CLint (μL/min/10 ⁶ cells) |
|------------|-------------------------|-------------|---------|------------------------------------|--|
| 7d | 2 | 98.8 | 3.9 | > 300.0 | 231.3 |
| 15b | 0.5 | 98.8 | 3.6 | > 300.0 | 48.9 |
| 17g | < 0.5 | 99.5 | 4.7 | > 300.0 | 183.4 |
| 17h | < 0.3 | 99.3 | > 3.2 | > 300.0 | 91.2 |
| 17j | < 0.1 | 99.4 | 4.8 | > 300.0 | 243.4 |
| 38a | 0.9 | 99.1 | 3.8 | 174.9 | 117.6 |
| 38b | 1 | 98.6 | 3.6 | 197.4 | 150.1 |
| 39a | 4 | 95.5 | > 3.4 | > 300.0 | 36.5 |
| 39b | 4 | 95.5 | > 3.4 | > 300.0 | 36.5 |
| 42a | 0.2 | 99.7 | 4 | 89.7 | 52.2 |

Biological profile - Having selected **42a** as the lead compound, full biological profiling was undertaken to establish its pharmacokinetic and toxicological profile in addition to its activity against slow-growing (Wayne assay) and MDR-resistant Mtb (Table 15).

Table 15. Biological profile of **42a**.

| | |
|---|--------------------------|
|  <p style="text-align: center;">42a</p> | |
| <i>In vitro</i> anti-tuberculosis activity | |
| Replicating sensitive Mtb IC ₅₀ (μM) | 0.525 |
| Replicating sensitive Mtb IC ₉₀ (μM) | 1.10 |
| Dormant (Wayne Model) Mtb IC ₉₀ (μM) | 0.076 |
| MDR Mtb (05TB42059) IC ₅₀ (μM) | 0.140 |
| MDR Mtb (DQ707(S315N kat G)) IC ₅₀ (μM) | 0.548 |
| <i>In vitro</i> DMPK | |
| Microsomal Turnover (h, m, r) T _{1/2} (min) | h-72.8, m-114.9, r-61.6 |
| Microsomal Cl _{int} (h, m, r) (μL/min/mg) | h-9.52, m-6.03, r-11.25 |
| Caco-2 permeability (cm ⁻¹ /s) A to B | 10.00 x 10 ⁻⁶ |
| Caco-2 permeability (cm ⁻¹ /s) B to A | 9.8 x 10 ⁻⁶ |
| Stability in plasma (r,h) T _{1/2} (min) | r->180, h->180 |
| Human % PPB | 97.30 |
| Solubility (μg/mL) pH1, pH7.4, CM | <1, <1, 55 |
| CYP2C8 Inhibition (% at 10 μM) | 38 |
| CYP2C9 Inhibition (% at 10 μM) | 0 |
| CYP2D6 Inhibition (% at 10 μM) | 0 |
| CYP3A4 Inhibition (% at 10 μM) | 0 |
| CYP3A5 Inhibition (% at 10 μM) | 0 |
| <i>In vitro</i> toxicity | |
| HEPG2 IC ₅₀ GLU (μM) | >100 |
| TI | >190 |
| hERG IC ₅₀ (μM) | >25 |
| Ames | -ve |

42a demonstrated comparable activity against all tested strains of sensitive and MDR Mtb as well as having good potency against dormant, non-replicating TB. It demonstrated a suitable *in vitro* DMPK and toxicity profile to undergo *in vivo* pharmacokinetic analysis.

Pharmacokinetics - the pharmacokinetic profile of **42a** can be seen in Figure 7 and Table 16. Analysis of data from the parent compound indicated solubility limited absorption as the PK did not increase linearly with dose from 10 mg/kg to 50 mg/kg. At this point the acetate pro-drug strategy was deployed in an attempt to improve exposure.

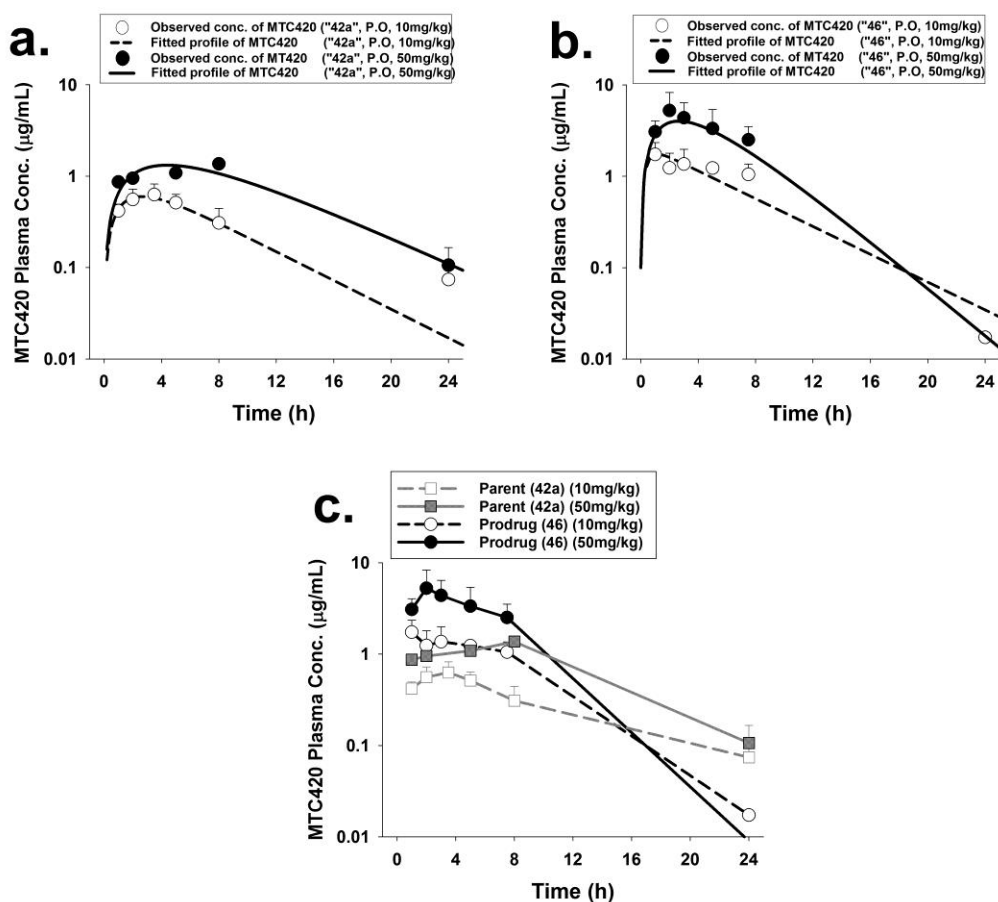


Figure 7. Pharmacokinetics after oral dosing of **42a** (a.), **46** (b.) and an overlay of both (c.)

Table 16. Pharmacokinetic parameters for **42a** and **46**.

| Parent 42a | | | | Prodrug 46 * | |
|----------------------------|----------|---------|---------|---------------------|---------|
| Dose (mg/kg) | 0.5 (iv) | 10 (po) | 50 (po) | 10 (po) | 50 (po) |
| T _{1/2} (h) | 1.48 | 3.8 | 4.2 | 3.9 | 2.3 |
| CL (L/h/kg) | 0.524 | - | - | - | - |
| V _{ss} (L/kg) | 0.291 | - | - | - | - |
| C _{max} (µg/mL) | - | 0.61 | 1.4 | 1.7 | 4.0 |
| AUC (mg.h/L) | 0.964 | 5.4 | 16.5 | 12.3 | 29.6 |
| Oral Bioavailability (% F) | N/A | 28.0 | 17.1 | 63.8 | 30.7 |

*These two studies were dosed with prodrug **46** orally, and measured for the parent **42a** in plasma.

Initial findings with both the 10 mg/kg and 50 mg/kg dose of pro-drug demonstrated a significant increase in overall exposure as indicated by a significantly increased AUC, C_{max} accompanied with increased bioavailability.

Metabolite ID work was undertaken to establish the metabolic activity exerted upon **46** (Figure 8 and Table 17).

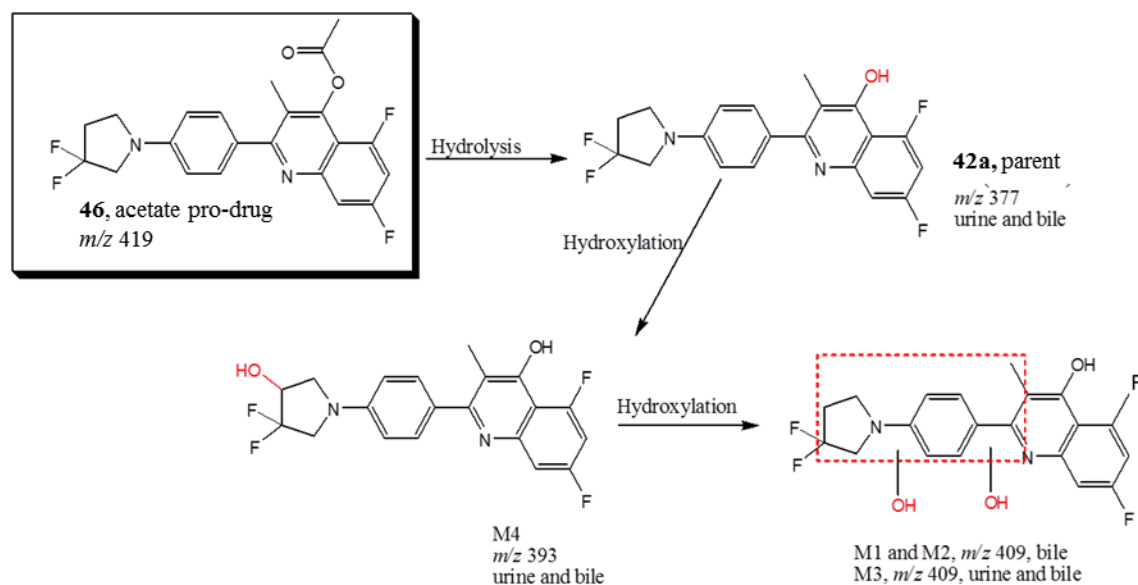


Figure 8. Metabolic pathways of pro-drug **46** in SD rat urine and bile.

Table 17. Identified metabolites of pro-drug **46** in SD rat urine and bile (MS)

| Peak ID | Mass Shift | Found m/z | Biotransformation | R.T(min) | Relative MS Abundance | |
|---------------------|------------|-------------|------------------------------|----------|-----------------------|----------|
| | | | | | Bile | Urine |
| 46 | 0 | 419 | Parent | 14.3 | ND | 1.85E+07 |
| M1 | -10 | 409 | Hydrolysis/ Hydroxylation | 8.6 | 5.89E+06 | ND |
| M2 | -10 | 409 | Hydrolysis/ Hydroxylation | 9.2 | 4.21E+06 | ND |
| M3 | -10 | 409 | Hydrolysis/ Hydroxylation | 9.9 | 5.92E+07 | 2.61E+07 |
| M4 | -26 | 393 | Hydrolysis/ Hydroxylation | 10.1 | 2.12E+07 | 3.80E+06 |
| M5 - 42a | -42 | 377 | Hydrolysis | 11.4 | 5.36E+06 | 6.12E+06 |

In the study, five metabolites were detected in the urine and bile of SD rats dosed with **46**. These metabolites were named as M1 through to M5 based on their eluting time under HPLC conditions. Among the five metabolites, M1, M2 and M3 were identified as di-hydroxy **42a**; M4 was identified as hydroxylated **42a**; M5 was identified as active drug **42a**. Location of the hydroxyl groups was established through mass spectrometry fragmentation patterns (see supporting information). M3 to M5 were detected both in urine and bile samples, M1 and M2 were only detected in the bile sample.

The presence of the pro-drug in the rat urine indicates that the pro-drug does not completely break down to its active metabolites as predicted. As the plasma levels obtained are a measure of

parent drug only, they are not a true representation of the drug levels present. Studies are currently underway to establish if a more suitable pro-drug can be synthesised that will resolve the issue and provide a compound suitable for *in vivo* efficacy testing.

CONCLUSIONS

To conclude, a 3-6 step synthesis of a range of 2-mono aryl amine 3-methyl quinolones with potent anti-tuberculosis activity has been reported. Compounds have been developed that are metabolically stable and have a good pharmacokinetic and toxicological profile. Importantly, the lead compound **42a** demonstrates equipotent activity against all drug sensitive and multi-drug resistant strains of Mtb tested. Work continues to develop a suitable pro-drug to embark on *in vivo* efficacy studies.

EXPERIMENTAL SECTION

Chemistry

All reactions that employed moisture sensitive reagents were performed in dry solvent under an atmosphere of nitrogen in oven dried glassware. All reagents were purchased from Sigma Aldrich or Alfa Aesar chemical companies, and were used without purification. Thin layer chromatography (TLC) was carried out on Merck silica gel 60 F-254 plates and U.V. inactive compounds were visualised using iodine or anisaldehyde solution. Flash column chromatography was performed on ICN Ecochrom 60 (32-63 mesh) silica gel eluting with various solvent mixtures and using an air line to apply pressure. NMR spectra were recorded on a Bruker AMX 400 (^1H , 400 MHz; ^{13}C , 100 MHz) spectrometer. Chemical shifts are described on parts per million (δ) downfield from an internal standard of trimethylsilane. Mass spectra were recorded on a VG analytical 7070E

machine and Fisons TRIO spectrometers using electron ionisation (EI) and chemical ionisation (CI). The optical rotation of the products were determined on Perkin Elmer Polarimeter (Model: 343Plus), and data was collected and processed by Expert Read 1.00.02 software. All compounds were found to be >95% pure by HPLC unless specified below. See supporting information for experimental methods and data relating to all intermediates.

Purity determination was performed by HPLC analysis using Agilent 1200 solvent delivery system. The HPLC methods used the following conditions: Knauer Eurospher 100-5 C18(250 mm X 4.6 mm) at 25°C with 1.5 mL/min flow rate; Method A: 90% acetonitrile containing 0.05% trifluoroacetic acid and 10% water containing 0.05% trifluoroacetic acid; Method B: 80% methanol and 20% acetonitrile.

General procedure for the preparation quinolones 1, 2, 7a-k, 17a-l, 21a-g, 24, 32a-g, 38a-j, 42a-b and 45a-g. Trifluoromethanesulfonic acid (26 μ L, 0.31 mmol, 0.2 eq) was added to oxazoline **4** (1.54 mmol) and the respective ketone (1.54 mmol, 1eq) in anhydrous n-butanol (10 mL). The mixture was heated to 130°C for 24 h (followed by tlc). The reaction was cooled and the solvent removed under reduced pressure. Sat. NaHCO₃ (aq) was added and the resulting aqueous solution was extracted with ethyl acetate (x 3), the combined organic layers were washed with water and brine, dried over MgSO₄, filtered and concentrated to a yellow solid. The crude product was triturated with diethyl ether to give the desired quinolone. In cases where trituration was not possible compounds were purified by flash column chromatography.

Preparation of 3-methyl-2-(4-(piperidin-1-yl)phenyl)quinolin-4(1H)-one 1. Light yellow powder (Yield 23%); m.p 290-292 °C; ¹H NMR (400MHz, CDCl₃), δ _H 8.46 (s, 1H, NH), 8.35 (d, 1H, J = 8.1 Hz, Ar), 7.59-7.52 (m, 1H, Ar), 7.36 (d, 2H, J = 8.7 Hz, Ar), 7.30 (dd, 2H, J = 15.1 H, 7.2 Hz,

Ar), 6.96 (d, 2H, J = 8.7 Hz, Ar), 2.10 (3H, CH₃), 1.78-1.61 (m, 10H, CH₂); ¹³C NMR (100MHz, CDCl₃), δ_C 179.1, 152.9, 148.0, 139.4, 131.8, 129.9, 126.7, 125.5, 124.0, 123.5, 117.4, 116.5, 115.6, 50.0, 26.0, 13.0; MS (ES+), [M + H]⁺ (100), 319.2, HRMS calculated for 319.1810 C₂₁H₂₃N₂O, found 319.1808; Anal. C₂₁H₂₂N₂O requires C 79.21%, H 6.96%, N 8.80%, found C 78.83%, H 6.85%, N 8.42%.

Preparation of 7-methoxy-3-methyl-2-(4-(piperidin-1-yl)phenyl)quinolin-4(1H)-one 2. Orange powder (Yield 36%); m.p 278-280 °C; ¹H NMR (400MHz, CDCl₃), δ_H 10.09 (s, 1H, NH), 8.16 (d, 1H, J = 8.5 Hz, Ar), 7.39 (d, 2H, J 0 8.9 Hz, Ar), 7.10 (d, 2H, J = 8.9 Hz, Ar), 6.92 (dd, 2H, J = 8.5 Hz, 2.6 Hz, Ar), 3.89 (s, 3H, OCH₃), 3.33-3.28 (m, 2H, CH₂), 2.06 (s, 3H, CH₃), 1.80-1.61 (m, 6H, CH₂); ¹³C NMR (100MHz, CDCl₃), δ_C 176.4, 161.8, 152.8, 129.5, 126.5, 124.7, 115.3, 114.7, 114.3, 97.7, 54.7, 25.3, 24.1, 11.4; MS (ES+), [M + H]⁺ (100), 348.2, HRMS calculated for 348.1916 C₂₂H₂₅N₃O, found 348.2002; Anal. C₂₂H₂₄N₂O₂ requires C 75.83%, H 6.94%, N 8.04%, found C 75.47%, H 6.83%, N 7.61%.

Preparation of 6-fluoro-3-methyl-2-(4-(piperidin-1-yl)phenyl)quinolin-4(1H)-one 7a. Orange powder (Yield 26%); m.p 328-330 °C ¹H NMR (400MHz, DMSO), δ_H 11.53 (s, 1H, NH), 7.71 (ddd, 1H, J = 13.9 Hz, 9.3 Hz, 3.9 Hz, Ar), 7.51 (ddd, 1H, J 9.1 Hz, 8.4 Hz, 3.0 Hz, Ar), 7.38 (d, 2H, J = 8.9 Hz, Ar), 7.07 (d, 2H, J = 8.9 Hz, Ar), 3.30-3.26 (m, 4H, CH₂), 1.95 (s, 3H, CH₃), 1.66-1.55 (m, 6H, CH₂); ¹³C NMR (100MHz, DMSO), δ_C 176.2, 157.1, 152.2, 148.6, 136.6, 130.2, 124.3, 121.2, 120.4, 115.0, 113.9, 109.1, 49.1, 25.3, 24.3, 12.8; MS (ES+), [M + H]⁺ (100), 337.2, HRMS calculated for 337.1716 C₂₁H₂₂N₂OF, found 337.1728; Anal. C₂₁H₂₁N₂OF requires C 74.98%, H 6.29%, N 8.33%, found C 74.51%, H 6.07%, N 8.04%.

Preparation of 6,7-dimethoxy-3-methyl-2-(4-(piperidin-1-yl)phenyl)quinolin-4(1H)-one 7b. Very pale yellow solid (Yield 28%); ¹H NMR (400 MHz, DMSO) δ_H 11.24 (s, 1H, NH), 7.45 (s, 1H,

Ar), 7.36 (d, $J = 8.8$ Hz, 2H, Ar), 7.16 – 6.98 (m, 3H, Ar), 3.83 (s, 3H, OCH₃), 3.82 (s, 3H, OCH₃), 3.29 – 3.25 (m, 4H, CH₂), 1.93 (s, 3H, CH₃), 1.69 – 1.53 (m, 6H, CH₂); ¹³C NMR (101 MHz, DMSO) δ_c 175.90 (C=O), 152.89, 152.05, 146.82, 146.54, 135.51, 130.19, 124.73, 117.34, 114.98, 113.15, 104.50, 99.38, 55.86 (OCH₃), 55.79 (OCH₃), 49.20, 25.35, 24.32, 12.86 (CH₃); HRMS (ESI) C₂₃H₂₇N₂O₃ [M+H]⁺ requires 379.2022, found 379.2012 (100%); Anal. C₂₃H₂₆N₂O₃ requires C 72.99%, H 6.92%, N 7.40%, found C 71.98%, H 6.96%, N 6.96%.

Preparation of 6-chloro-7-methoxy-3-methyl-2-(4-(piperidin-1-yl)phenyl)quinolin-4(1H)-one 7c.

White solid (Yield 35%); m.p. >300°C. ¹H NMR (400 MHz, DMSO) δ_H 11.42 (s, 1H, NH), 8.02 (s, 1H, Ar), 7.38 (d, $J = 8.8$ Hz, 2H, Ar), 7.21 (s, 1H, Ar), 7.07 (d, $J = 8.9$ Hz, 2H, Ar), 3.91 (s, 3H, OCH₃), 3.31 – 3.22 (m, 4H, CH₂), 1.93 (s, 3H, CH₃), 1.71 – 1.52 (m, 6H, CH₂); ¹³C NMR (101 MHz, DMSO) δ_c 175.63 (C=O), 156.74, 152.17, 148.16, 140.13, 130.21, 126.09, 124.18, 118.08, 117.91, 114.89, 114.25, 100.13, 56.59 (OCH₃), 49.10, 25.32, 24.32, 12.70 (CH₃); HRMS (ESI) C₂₂H₂₄N₂O₂³⁵Cl [M+H]⁺ requires 383.1526, found 383.1513 (100%), C₂₂H₂₄N₂O₂³⁷Cl [M+H]⁺ requires 385.1497, found 385.1501 (34%). Anal. C₂₂H₂₃N₂O₂Cl requires C 69.01%, H 6.05%, N 7.32%, found C 68.98%, H 6.04%, N 7.23%.

Preparation of 6-fluoro-7-methoxy-3-methyl-2-(4-(piperidin-1-yl)phenyl)quinolin-4(1H)-one 7d.

White solid (Yield 41%) ¹H NMR (400 MHz, DMSO) δ_H 11.39 (s, 1H, NH), 7.71 (d, $J = 11.9$ Hz, 1H, Ar), 7.37 (d, $J = 8.7$ Hz, 2H, Ar), 7.24 (d, $J = 7.5$ Hz, 1H, Ar), 7.07 (d, $J = 8.8$ Hz, 2H, Ar), 3.90 (s, 3H, OCH₃), 3.30 – 3.19 (m, 4H, CH₂), 1.92 (s, 3H, CH₃), 1.74 – 1.48 (m, 6H, CH₂); ¹³C NMR (101 MHz, DMSO) δ_c 175.94 (C=O), 152.15, 151.00, 150.87, 150.35, 147.88, 137.55, 130.20, 124.30, 114.93, 113.57, 110.03, 101.12, 56.36 (OCH₃), 49.13, 25.33, 24.32, 12.70 (CH₃); HRMS (ESI) C₂₂H₂₄N₂O₂F [M+H]⁺ requires 367.1822, found 367.1818. Anal. C₂₂H₂₃N₂O₂F requires C 72.11%, H 6.33%, N 7.64%, found C 71.95%, H 6.45%, N 7.37%.

Preparation of 5,7-dimethoxy-3-methyl-2-(4-(piperidin-1-yl)phenyl)quinolin-4(1H)-one 7e. White solid (Yield 32%); m.p. 264 – 265°C. ¹H NMR (400 MHz, DMSO) δ_H 10.93 (s, 1H, NH), 7.33 (d, *J* = 8.7 Hz, 2H, Ar), 7.05 (d, *J* = 8.7 Hz, 2H, Ar), 6.64 (d, *J* = 2.2 Hz, 1H, Ar), 6.25 (d, *J* = 2.1 Hz, 1H, Ar), 3.78 (s, 3H, OCH₃), 3.77 (s, 3H, OCH₃), 3.32 – 3.11 (m, 4H, CH₂), 1.82 (s, 3H, CH₃), 1.70 – 1.48 (m, 6H, CH₂); ¹³C NMR (101 MHz, DMSO) δ_C 176.49 (C=O), 161.75, 161.03, 152.02, 145.53, 143.94, 130.15, 124.47, 115.49, 114.98, 109.24, 94.23, 91.57, 55.97 (OCH₃), 55.48 (OCH₃), 49.22, 25.35, 24.32, 12.82 (CH₃); HRMS (ESI) C₂₃H₂₇N₂O₂ [M+H]⁺ requires 379.2022, found 379.2007. Anal. C₂₃H₂₆N₂O₂ requires C 72.99%, H 6.92%, N 7.40%, found C 72.13%, H 6.88%, N 7.03%.

Preparation of 5,7-difluoro-3-methyl-2-(4-(piperidin-1-yl)phenyl)quinolin-4(1H)-one 7f. Off white solid (0.25 g, 35 %); mp 305-306 °C; ¹H NMR (400 MHz, DMSO) δ 11.50 (bs, 1H), 7.37 (d, *J* = 8.8 Hz, 2H), 7.15 (d, *J* = 9.2 Hz, 1H), 7.08 (d, *J* = 8.9 Hz, 2H), 6.98 (t, *J* = 9.6 Hz, 1H), 3.30 (m, 4H), 1.88 (s, 3H), 1.61 (m, 6H); ¹³C NMR (100 MHz, CDCl₃) δ_C 175.2, 152.1, 148.6, 130.2, 116.1, 114.9, 100.2, 49.2, 25.3, 24.3, 12.6; MS (ES⁺) *m/z* 355 (M + H)⁺ HRMS calculated for 355.1622 C₂₁H₂₁N₂OF₂, found 355.1625; Purity HPLC 95% (method A) R_t = 2.34 min.

Preparation of 7-fluoro-3-methyl-2-(4-(piperidin-1-yl)phenyl)quinolin-4(1H)-one 7g. Off white solid (0.15 g, 35 %); mp 343-345 °C; ¹H NMR (400 MHz, DMSO) δ 8.14 (dd, *J* = 9.0, 6.6 Hz, 1H), 7.38 (d, *J* = 8.8 Hz, 2H), 7.30 (dd, *J* = 10.5, 2.3 Hz, 1H), 7.10 (m, 1H), 7.05 (d, *J* = 8.8 Hz, 2H), 3.28 (m, 4H), 1.94 (s, 3H), 1.62 (m, 6H); ¹³C NMR (100 MHz, DMSO) δ_C not soluble in DMSO; MS (ES⁺) *m/z* 337 (M + H)⁺ HRMS calculated for 337.1716 C₂₁H₂₂N₂OF, found 337.1722; Purity HPLC 97% (Method B) R_t = 2.44 min.

Preparation of 7-chloro-3-methyl-2-(4-(piperidin-1-yl)phenyl)quinolin-4(1H)-one 7h. Off white solid (0.17 g, 37 %); mp 342-343 °C; ¹H NMR (400 MHz, DMSO) δ 8.08 (d, *J* = 8.7 Hz, 1H), 7.59

(s, 1H), 7.40 (d, J = 8.8 Hz, 2H), 7.18 (dd, J = 8.7, 2.0 Hz, 1H), 7.04 (d, J = 8.8 Hz, 2H), 3.08 (m, 4H), 1.95 (s, 3H), 1.61 (m, 6H); ^{13}C NMR (100 MHz, CDCl_3) δ_{C} not soluble in DMSO; MS (ES^+) m/z 353 ($\text{M} + \text{H}$) $^+$ HRMS calculated for 353.1425 $\text{C}_{21}\text{H}_{22}\text{N}_2\text{O}^{35}\text{Cl}$, found 353.1421; Purity HPLC 97% (Method A) R_t = 2.07 min.

Preparation of 2-(4-(4-fluoropiperidin-1-yl)phenyl)-3-methylquinolin-4(1H)-one 7i. White solid (0.18 g, 36 %). ^1H NMR (400 MHz, DMSO) 8.10 (d, J = 8.8 Hz, 1H), 7.57 (m, 2H), 7.40 (d, J = 8.8 Hz, 2H), 7.24 (dd, J = 7.2, 6.8 Hz, 1H), 7.11 (d, J = 8.8 Hz, 2H), 4.88 (d, J = 48.8 Hz, 1H), 3.24 (m, 4H), 2.03 (m, 2H), 1.95 (s, 3H), 1.80 (m, 2H); ^{13}C NMR (100 MHz, DMSO) δ_{C} 176.4, 150.7, 130.6, 130.0, 124.9, 123.3, 122.1, 119.0, 114.8, 113.8, 89.4, 87.8, 44.6, 44.5, 30.5, 30.3, 12.6; MS (ES^+) m/z 337 ($\text{M} + \text{H}$) $^+$ HRMS calculated for 337.1716 $\text{C}_{21}\text{H}_{22}\text{N}_2\text{OF}$, found 337.1720; Purity HPLC 96% (Method A) R_t = 2.21 min.

Preparation of 2-(4-(4-fluoropiperidin-1-yl)phenyl)-7-methoxy-3-methylquinolin-4(1H)-one 7j. Yellow solid (Yield 43%) ^1H NMR (400 MHz, DMSO) δ 11.26 (s, 1H, NH), 8.00 (d, J = 8.9 Hz, 1H, Ar), 7.39 (d, J = 8.6 Hz, 2H, Ar), 7.12 (d, J = 8.6 Hz, 2H, Ar), 7.05 (d, J = 2.1 Hz, 1H, Ar), 6.88 (dd, J = 8.9, 2.2 Hz, 1H, Ar), 5.02 – 4.77 (m, 1H, CH), 3.82 (s, 3H, OCH_3), 3.57 – 3.44 (m, 2H, CH_2), 3.32 – 3.20 (m, 2H, CH_2), 2.13 – 1.95 (m, 2H, CH_2), 1.91 (s, 3H, CH_3), 1.86 – 1.71 (m, 2H, CH_2); ^{13}C NMR (101 MHz, DMSO) δ 176.78 (C=O), 161.89, 151.28, 147.80, 141.66, 130.39, 127.22, 125.12, 117.98, 115.22, 114.02, 113.14, 99.23, 89.01 (d, J = 169.4 Hz, C-F), 55.74, 44.87 (d, J = 6.8 Hz), 30.84 (d, J = 19.0 Hz), 12.78 (CH_3); HRMS (ESI) $\text{C}_{22}\text{H}_{24}\text{N}_2\text{O}_2\text{F}$ [$\text{M} + \text{H}$] $^+$ requires 367.1822, found 367.1836. Anal. $\text{C}_{22}\text{H}_{23}\text{N}_2\text{O}_2\text{F}$ requires C 72.11%, H 6.33%, N 7.64%, found C 71.32%, H 6.34%, N 7.46%.

Preparation of 5,7-difluoro-2-(4-(4-fluoropiperidin-1-yl)phenyl)-3-methylquinolin-4(1H)-one 7k. White solid (29%); m.p > 320 °C. ^1H NMR (400 MHz, DMSO) 11.51 (s, 1H), 7.40 (m, 2H), 7.15

(m, 3H), 7.00 (m, 1H), 3.49 (m, 2H), 3.24 (m, 2H), 2.0 (m, 2H), 1.89 (s, 3H), 1.75 (m, 2H); ^{13}C NMR (100 MHz, DMSO) δ_{C} not soluble in DMSO; MS (ES^+) m/z 373 ($\text{M} + \text{H}$) $^+$ HRMS calculated for 373.1519 $\text{C}_{21}\text{H}_{20}\text{N}_2\text{OF}_3$, found 373.7528; Purity HPLC 97% (Method A) R_t = 2.18 min.

General procedure for the preparation of compounds 14a-c. To a solution of ketone **13** (0.24 mmol) in anhydrous 1,4-dioxane (8 ml) was added ground sodium hydroxide (30 mg, 0.75 mmol, 3 equiv). The mixture was allowed to reflux at 110°C for 5 h. The solution was cooled to room temperature and acidified by addition of 2N hydrochloric acid. The solid was filtered and washed with water, followed by ethyl acetate and dried.

Preparation of 2-(4-(piperidin-1-yl)phenyl)quinolin-4(1H)-one 14a. White solid (0.25 g, 70 %). m.p. 350 °C; ^1H NMR (400 MHz, DMSO) δ 11.42 (bs, 1H), 8.07 (d, J = 8.0 Hz, 1H), 7.76 (d, J = 8.3 Hz, 1H), 7.71 (d, J = 8.6 Hz, 2H), 7.64 (dd, J = 8.3, 7.0 Hz, 1H), 7.30 (dd, J = 8.3, 7.0 Hz, 1H), 7.07 (d, J = 8.8 Hz, 2H), 6.29 (s, 1H), 3.33 (m, 4H), 1.19 (m, 6H); ^{13}C NMR (100 MHz, DMSO) δ_{C} not soluble in DMSO; MS (ES^+) m/z 305 ($\text{M} + \text{H}$) $^+$ HRMS calculated for 305.1654 $\text{C}_{20}\text{H}_{21}\text{N}_2\text{O}$, found 305.1662; Anal. $\text{C}_{20}\text{H}_{20}\text{N}_2\text{O}$ requires C 78.92%, H 6.62%, N 9.20%, found C 78.67%, H 6.55%, N 8.89%.

Preparation of 7-methoxy-2-(4-(piperidin-1-yl)phenyl)quinolin-4(1H)-one 14b. White solid (0.065 g, 41 %). m.p. 350 °C; ^1H NMR (400 MHz, DMSO) δ 11.30 (bs, 1H), 7.96 (d, J = 8.9 Hz, 1H), 7.69 (d, J = 8.7 Hz, 2H), 7.23 (d, J = 2.3 Hz, 1H), 7.07 (d, J = 8.9 Hz, 2H), 6.89 (dd, J = 8.0, 4.0 Hz, 1H), 6.21 (s, 1H), 3.86 (s, 3H), 3.34 (m, 4H), 1.60 (m, 6H); ^{13}C NMR (100 MHz, DMSO) δ_{C} not soluble in DMSO; MS (ES^+) m/z 335 ($\text{M} + \text{H}$) $^+$ HRMS calculated for 335.1760 $\text{C}_{21}\text{H}_{23}\text{N}_2\text{O}_2$, found 335.1761; Purity HPLC 96% (method A) R_t = 1.81 min.

Preparation of 2-(4-(4-fluoropiperidin-1-yl)phenyl)-7-methoxyquinolin-4(1H)-one 14c. Yellow solid (Yield 68%). ^1H NMR (400 MHz, DMSO) δ_{H} 13.70 (s, 1H, NH), 8.18 (d, J = 9.2 Hz, 1H,

Ar), 7.88 (d, $J = 9.0$ Hz, 2H, Ar), 7.58 (d, $J = 2.3$ Hz, 1H, Ar), 7.34 (dd, $J = 9.2, 2.4$ Hz, 1H, Ar), 7.31 – 7.19 (m, 3H, Ar), 4.93 (dtt, $J = 48.9, 7.0, 3.4$ Hz, 1H, CH), 3.98 (s, 3H, OCH₃), 3.71 – 3.58 (m, 2H, CH₂), 3.51 – 3.37 (m, 2H, CH₂), 2.10 – 1.88 (m, 2H, CH₂), 1.87 – 1.68 (m, 2H, CH₂); HRMS (ESI) C₂₁H₂₂N₂O₂F [M+H]⁺ requires 353.1665, found 353.1667; Anal. C₂₁H₂₁N₂O₂F requires C 71.57%, H 6.01%, N 7.95%, found C 71.12%, H 5.93%, N 7.71%.

General procedure for the preparation of compounds 15a-d. Quinolone **14** (0.33 mmol) was added to MeOH (20 mL), 2M NaOH (4 mL) and water (4 mL). Sodium dichloroisocyanurate (36 mgs, 0.17 mmol, 0.5 eq) was added at room temperature and the resultant light orange solution was allowed to stir overnight. The solvent was removed *in vacuo* and the residue was dissolved in EtOAc (100 mL), followed by washing with water (50 mL) and brine (50 mL). The crude product was purified by column chromatography (eluting with 100 % EtOAc) to afford the desired product.

Preparation of 3-chloro-2-(4-(piperidin-1-yl)phenyl)quinolin-4(1H)-one 15a. White solid (40 mgs, 40 %); ¹H NMR (400 MHz, DMSO) δ 12.01 (bs, 1H), 8.15 (d, $J = 7.9$ Hz, 1H), 7.69 (m 2H), 7.52 (d, $J = 8.7$ Hz, 2H), 7.38 (m, 1H), 7.09 (d, $J = 8.8$ Hz, 2H), 3.33 (m, 4H), 1.61 (m, 6H); ¹³C NMR (100 MHz, DMSO) δ_c 171.7, 152.5, 148.7, 139.3, 132.2, 130.7, 125.4, 124.0, 123.8, 122.1, 118.9, 114.5, 113.2, 48.9, 25.3, 24.3; MS (ES⁺) m/z 339 (M + H)⁺ HRMS calculated for 339.1264 C₂₀H₂₀N₂O³⁵Cl, found 339.1252; Purity HPLC 98% (method A) R_t = 2.13 min.

Preparation of 3-chloro-7-methoxy-2-(4-(piperidin-1-yl)phenyl)quinolin-4(1H)-one 15b. White solid (27 mgs, 61 %); ¹H NMR (400 MHz, DMSO) δ 11.82 (bs, 1H), 8.04 (d, $J = 9.0$ Hz, 1H), 7.52 (d, $J = 8.8$ Hz, 2H), 7.11 (m, 3H), 6.99 (dd, $J = 9.2, 2.4$ Hz, 1H), 3.85 (s, 3H), 3.33 (m, 4H), 1.61 (m, 6H); ¹³C NMR (100 MHz, DMSO) δ_c 162.3, 148.1, 141.1, 130.6, 127.3, 118.2, 114.7, 114.2, 112.9, 99.7, 55.8, 49.1, 25.2, 24.2; MS (ES⁺) m/z 369 (M + H)⁺ HRMS calculated for 369.1370 C₂₁H₂₂N₂O₂³⁵Cl, found 369.1375; Purity HPLC 99% (method A) R_t = 1.83 min.

Preparation of 3-chloro-2-(4-(4-fluoropiperidin-1-yl)phenyl)-7-methoxyquinolin-4(1H)-one 15c.

Yellow solid (Yield 52%). MP 304 – 306°C. ¹H NMR (400 MHz, DMSO) δ_H 11.86 (s, 1H, NH), 8.03 (d, *J* = 9.0 Hz, 1H, Ar), 7.52 (d, *J* = 8.8 Hz, 2H, Ar), 7.14 (d, *J* = 8.9 Hz, 2H, Ar), 7.10 (d, *J* = 2.3 Hz, 1H, Ar), 6.98 (dd, *J* = 9.0, 2.4 Hz, 1H, Ar), 4.90 (dtt, *J* = 21.4, 7.3, 3.6 Hz, 1H, CH), 3.84 (s, 3H, OCH₃), 3.63 – 3.46 (m, 2H), 3.34 – 3.19 (m, 2H), 2.13 – 1.90 (m, 2H), 1.85 – 1.58 (m, 2H); ¹³C NMR (101 MHz, DMSO) δ_C 171.31 (C=O), 162.33 (C-O), 151.61, 148.08, 141.06, 130.67, 127.30, 122.71, 118.20, 114.66, 114.20, 112.89, 99.63, 88.89 (d, *J* = 169.5 Hz, C-F), 55.81, 44.53 (d, *J* = 6.8 Hz), 30.69 (d, *J* = 19.1 Hz); HRMS (ESI) C₂₁H₂₁N₂O₂F³⁵Cl [M+H]⁺ requires 387.1276, found 387.1287. Anal. C₂₁H₂₀N₂O₂FCl requires C 65.20%, H 5.21%, N 7.24%, found C 64.90%, H 5.35%, N 6.95%.

Preparation of 3-chloro-2-(4-(4-fluoropiperidin-1-yl)phenyl)quinolin-4(1H)-one 15d.

Light yellow solid (0.19 g, 58 %). ¹H NMR (400 MHz, DMSO) 12.05 (bs, 1H), 8.15 (d, *J* = 8.0 Hz, 1H), 7.70 (d, *J* = 4.0 Hz, 2H), 7.54 (d, *J* = 8.8 Hz, 2H), 7.38 (m, 1H), 7.15 (d, *J* = 8.8 Hz, 2H), 4.89 (d, *J* = 48.0 Hz, 1H), 3.53 (m, 2H), 3.30 (m, 2H), 1.97 (m, 2H), 1.79 (m, 2H); ¹³C NMR (100 MHz, DMSO) δ_C 175.2, 150.8, 128.5, 127.9, 127.3, 124.6, 115.4, 103.9, 89.9, 88.2, 79.6, 66.7, 45.2, 45.1, 31.0, 30.8, 15.5; MS (ES⁺) *m/z* 357 (M + H)⁺ HRMS calculated for 357.1170 C₂₀H₁₉N₂O³⁵FCl, found 357.1159; Purity HPLC 95% (Method A) R_t = 2.15 min.

General procedure for the preparation of compounds 15e-f. Quinolone **14** (0.33 mmol) was added to DCM (15 mL) and MeOH (4 mL). NBS (58 mgs, 0.33 mmol) was added at room temperature and the resultant bright yellow solution was allowed to stir overnight. The solvent was removed *in vacuo* and the residue was dissolved in EtOAc (100 mL), followed by washing with water (50 mL) and brine (50 mL). The crude product was purified by column chromatography (eluting with 70 % EtOAc in *n*-hexanes) to afford the desired product.

Preparation of 3-bromo-2-(4-(piperidin-1-yl)phenyl)quinolin-4(1H)-one 15e. White solid (63 %); ^1H NMR (400 MHz, DMSO) δ 12.07 (bs, 1H), 8.15 (d, J = 8.1 Hz, 1H), 7.68 (m 2H), 7.49 (d, J = 8.8 Hz, 2H), 7.39 (ddd, J = 8.6, 7.9, 4.1 Hz, 1H), 7.08 (d, J = 8.8 Hz, 2H), 3.31 (m, 4H), 1.62 (m, 6H); ^{13}C NMR (100 MHz, DMSO) δ_{C} 172.1, 152.5, 150.4, 139.4, 132.3, 130.6, 125.6, 124.2, 124.0, 123.2, 118.8, 114.5, 105.5, 49.0, 25.3, 24.4; MS (ES^+) m/z 383 ($\text{M} + \text{H}$) $^+$ HRMS calculated for 383.0759 $\text{C}_{20}\text{H}_{20}\text{N}_2\text{O}^{79}\text{Br}$, found 383.0748; Purity HPLC 98% (Method A) R_t = 1.75 min.

Preparation of 3-bromo-2-(4-(4-fluoropiperidin-1-yl)phenyl)quinolin-4(1H)-one 15f. Light yellow solid (0.20g, 55 %). ^1H NMR (400 MHz, DMSO) 12.26 (bs, 1H), 8.15 (d, J = 8.8 Hz, 1H), 7.69 (m, 1H), 7.50 (d, J = 8.8 Hz, 2H), 7.39 (m, 2H), 7.14 (d, J = 8.8 Hz, 2H), 4.89 (d, J = 48.0 Hz, 1H), 3.53 (m, 2H), 3.30 (m, 2H), 1.97 (m, 2H), 1.79 (m, 2H). ^{13}C NMR (100 MHz, DMSO) δ_{C} 179.7, 150.3, 132.3, 130.2, 125.6, 124.5, 114.6, 105.6, 89.7, 88.1, 44.6, 44.5, 30.8, 15.5; MS (ES^+) m/z 401 ($\text{M} + \text{H}$) $^+$ HRMS calculated for 401.0665 $\text{C}_{20}\text{H}_{19}\text{N}_2\text{OF}^{79}\text{Br}$, found 401.0656; Purity HPLC 99% (Method A) R_t = 2.15 min.

Preparation of 5,7-difluoro-3-methyl-2-(4-(4-(trifluoromethyl)piperidin-1-yl)phenyl)quinolin-4(1H)-one 17a. White solid (32%); m.p. >350 °C. ^1H NMR (400 MHz, DMSO) 11.52 (s, 1H), 7.39 (m, 2H), 7.17 (m, 3H), 7.00 (m, 1H), 3.95 (m, 2H), 2.85 (m, 2H), 1.91 (m, 2H), 1.87 (s, 3H), 1.55 (m, 2H); ^{13}C NMR (100 MHz, DMSO) δ_{C} not soluble in DMSO; MS (ES^+) m/z 423 ($\text{M} + \text{H}$) $^+$ HRMS calculated for 423.1496 $\text{C}_{22}\text{H}_{20}\text{N}_2\text{OF}_5$, found 423.1483; Anal. $\text{C}_{22}\text{H}_{19}\text{N}_2\text{OF}_5$ requires C 62.56%, H 4.53%, N 6.63%, found C 62.49%, H 4.52%, N 6.62%.

Preparation of 5,7-difluoro-3-methyl-2-(4-(4-methylpiperidin-1-yl)phenyl)quinolin-4(1H)-one 17b. White solid (54%); m.p. decomposed at 310°C. NMR: ^1H (400 MHz, DMSO) δ 11.50 (s, 1H), 7.36 (d, J = 8.8 Hz, 2H), 7.16 (d, J = 10.0 Hz, 1H), 7.08 (d, J = 8.9 Hz, 2H), 7.00 (ddd, J = 12.0, 9.6, 2.4 Hz, 1H), 3.83 (d, J = 12.8 Hz, 2H), 2.76 (td, J = 12.5, 2.4 Hz, 2H), 1.87 (s, 3H), 1.70 (d, J

= 12.7 Hz, 2H), 1.63 – 1.49 (m, 1H), 1.21 (qd, $J = 12.7, 4.0$ Hz, 2H), 0.94 (d, $J = 6.5$ Hz, 3H); ^{13}C (101 MHz, DMSO) δ 175.37, 163.51, 160.76, 152.01, 147.65, 142.84, 130.21, 123.60, 116.33, 114.91, 110.49, 99.41, 98.81, 48.41, 33.55, 30.65, 22.18, 12.51. ES HRMS: m/z found 369.1792, $\text{C}_{22}\text{H}_{23}\text{N}_2\text{OF}_2$ requires 369.1778; Anal. $\text{C}_{22}\text{H}_{22}\text{N}_2\text{OF}_2$ requires C 71.72%, H 6.02%, N 7.60%, found C 71.66%, H 5.95%, N 7.52%.

Preparation of 2-(4-(6-azaspiro[2.5]octan-6-yl)phenyl)-5,7-difluoro-3-methylquinolin-4(1H)-one
17c. White solid (Yield 34%); m.p. > 300°C. ^1H NMR (400 MHz, DMSO) δ_{H} 11.51 (s, 1H, NH), 7.38 (d, $J = 8.7$ Hz, 2H), 7.16 (d, $J = 10.0$ Hz, 1H), 7.11 (d, $J = 8.8$ Hz, 2H), 7.00 (ddd, $J = 11.9, 9.6, 2.3$ Hz, 1H), 3.38 – 3.35 (m, 4H), 1.88 (s, 3H, CH_3), 1.53 – 1.36 (m, 4H), 0.35 (s, 4H); ^{13}C NMR (101 MHz, DMSO) δ 175.52, 163.75 (d, $J = 61.6$ Hz), 161.38 (d, $J = 77.0$ Hz), 152.14, 147.75, 142.80 (dd, $J = 14.7, 6.3$ Hz), 130.34, 123.72, 116.45, 115.22, 110.59 (d, $J = 2.4$ Hz), 99.60 (dd, $J = 24.9, 4.1$ Hz), 98.95 (dd, $J = 28.7, 25.6$ Hz), 48.40, 34.32, 18.15, 12.61, 11.59. HRMS (ESI) $\text{C}_{23}\text{H}_{22}\text{N}_2\text{OF}^{23}\text{Na}$ [$\text{M}+\text{Na}$] $^+$ requires 403.1598, found 403.1612. Anal. $\text{C}_{23}\text{H}_{22}\text{N}_2\text{OF}$ requires C 72.61%, H 5.83%, N 7.36%, found C 72.41%, H 5.91%, N 7.31%.

Preparation of 2-(4-(4,4-difluoropiperidin-1-yl)phenyl)-5,7-difluoro-3-methylquinolin-4(1H)-one
17d. White solid (0.30 g, 57 %). ^1H NMR (400 MHz, DMSO) 7.38 (d, $J = 8.8$ Hz, 2H), 7.10 (d, $J = 8.8$ Hz, 2H), 7.07 (m, 1H), 6.82 (dd, $J = 11.0, 10.6$ Hz, 1H), 3.43 (m, 4H), 2.07 (m, 4H), 1.88 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ_{C} 174.2, 149.5, 129.9, 122.8, 118.5, 115.3, 115.0, 45.3, 33.0, 32.8, 32.5, 12.6; MS (Cl^+) m/z 391 ($\text{M} + \text{H}$) $^+$ HRMS calculated for 391.1428 $\text{C}_{21}\text{H}_{19}\text{N}_2\text{OF}_4$, found 391.1430; Purity HPLC 95% (Method A) $R_t = 2.39$ min.

Preparation of 5,7-difluoro-2-(4-(3-fluoropiperidin-1-yl)phenyl)-3-methylquinolin-4(1H)-one
17e. Light brown solid (0.12 g, 27 %). ^1H NMR (400 MHz, DMSO) 11.49 (bs, 1H), 7.38 (d, $J = 8.8$ Hz, 2H), 7.10 (d, $J = 8.8$ Hz, 2H), 7.07 (m, 1H), 6.99 (dd, $J = 11.0, 10.6$ Hz, 1H), 4.82 (d, $J =$

48.8 Hz, 1H), 3.50-3.33 (m, 4H), 1.87 (s, 3H), 1.86-1.62 (m, 4H) ; ^{13}C NMR (100 MHz, DMSO) δ_{C} 175.2, 151.4, 147.1, 129.8, 123.6, 116.0, 114.6, 98.8, 88.1, 86.4, 51.8, 51.6, 47.3, 29.3, 29.1, 20.6, 20.5, 12.1; MS (EI^+) m/z 373 ($\text{M} + \text{H}$) $^+$ HRMS calculated for 373.1528 $\text{C}_{21}\text{H}_{20}\text{N}_2\text{OF}_3$, found 373.1524; Purity HPLC 97% (Method A) R_t = 2.42 min.

Preparation of 5,7-difluoro-3-methyl-2-(4-(3-methylpiperidin-1-yl)phenyl)quinolin-4(1H)-one 17f. White solid (45%). Melting point: 280~282°C. NMR: ^1H (400 MHz, DMSO) δ 11.50 (s, 1H), 7.36 (d, J = 8.8 Hz, 2H), 7.16 (d, J = 9.0 Hz, 1H), 7.07 (d, J = 8.9 Hz, 2H), 7.00 (ddd, J = 12.0, 9.6, 2.4 Hz, 1H), 3.77 (t, J = 11.6 Hz, 2H), 2.72 (td, J = 12.3, 2.9 Hz, 1H), 2.42 (dd, J = 12.4, 10.7 Hz, 1H), 1.87 (s, 3H), 1.82 – 1.48 (m, 4H), 1.09 (ddd, J = 23.5, 12.4, 3.9 Hz, 1H), 0.93 (d, J = 6.6 Hz, 3H). ^{13}C (101 MHz, DMSO) δ 175.37, 164.10, 161.50, 152.00, 147.66, 142.69, 130.22, 123.46, 116.33, 114.81, 110.59, 99.40, 98.79, 55.93, 48.45, 32.93, 30.35, 24.72, 19.58, 12.50. ES HRMS: m/z found 369.1772, $\text{C}_{22}\text{H}_{23}\text{N}_2\text{OF}_2$ requires 369.1778; Anal. $\text{C}_{22}\text{H}_{22}\text{N}_2\text{OF}_2$ requires C 71.72%, H 6.02%, N 7.60%, found C 71.76%, H 5.94%, N 7.58%.

Preparation of (R)-5,7-difluoro-3-methyl-2-(4-(3-methylpiperidin-1-yl)phenyl)quinolin-4(1H)-one 17g. White solid (43%). ^1H and ^{13}C NMR data is the same as the racemic analogue; ES HRMS: m/z found 369.1775, $\text{C}_{22}\text{H}_{23}\text{N}_2\text{OF}_2$ requires 369.1778; Anal. $\text{C}_{22}\text{H}_{22}\text{N}_2\text{OF}_2$ requires C 71.72%, H 6.02%, N 7.60%, found C 71.68%, H 6.06%, N 7.53%; the optical rotation was measured as $[\alpha]_{\text{D}}^{22} = +81.5^\circ \pm 0.9$ ($c=0.558\text{g}/100\text{ml}$ in MeOH).

Preparation of (S)-5,7-difluoro-3-methyl-2-(4-(3-methylpiperidin-1-yl)phenyl)quinolin-4(1H)-one 17h. White solid (40%). ^1H and ^{13}C NMR data is the same as the racemic analogue; ES HRMS: m/z found 369.1782, $\text{C}_{22}\text{H}_{23}\text{N}_2\text{OF}_2$ requires 369.1778; Anal. $\text{C}_{22}\text{H}_{22}\text{N}_2\text{OF}_2$ requires C 71.72%, H 6.02%, N 7.60%, found C 71.77%, H 6.0%, N 7.64%; the optical rotation was measured as $[\alpha]_{\text{D}}^{22} = -86.1^\circ \pm 0.7$ ($c=0.588\text{g}/100\text{ml}$ in MeOH).

Preparation of 5,7-difluoro-3-methyl-2-(4-(4-methylpiperazin-1-yl)phenyl)quinolin-4(1H)-one 17i. White solid (39%); m.p. >350 °C. ¹H NMR (400 MHz, DMSO) 11.50 (s, 1H), 7.89 (d, *J* = 9.0, 2H), 7.19 (m, 1H), 7.05 (m, 1H), 6.85 (d, *J* = 9.0, 2H), 3.35 (m, 4H), 2.55 (m, 4H), 2.31 (s, 3H), 1.90 (s, 3H); ¹³C NMR (100 MHz, DMSO) δ_C not soluble in DMSO; MS (ES⁺) *m/z* 370 (M + H)⁺ HRMS calculated for 370.1717 C₂₁H₂₂N₃OF₂, found 370.1731; Purity HPLC 99% (Method A) R_t = 1.59 min.

Preparation of 2-(4-(azepan-1-yl)phenyl)-5,7-difluoro-3-methylquinolin-4(1H)-one 17j. White solid (41%); m.p. >350 °C. ¹H NMR (400 MHz, DMSO) 11.45 (s, 1H), 7.31 (d, *J* = 8.8, 2H), 7.19 (m, 1H), 7.00 (m, 1H), 6.85 (d, *J* = 8.9, 2H), 3.55 (m, 4H), 1.92 (s, 3H), 1.75 (bs, 4H), 1.45 (bs, 4H); ¹³C NMR (100 MHz, DMSO) δ_C 175.4, 149.4, 147.8, 130.5, 120.5, 116.1, 110.8, 99.54, 98.9, 98.7, 49.1, 48.1, 47.9, 47.7, 47.5, 27.0, 26.6, 12.6; MS (ES⁺) *m/z* 369 (M + H)⁺ HRMS calculated for 369.1764 C₂₂H₂₃N₂OF₂, found 369.1778; Anal. C₂₂H₂₂N₂OF₂ requires C 71.72%, H 6.02%, N 7.60%, found C 71.36%, H 5.97%, N 7.39%.

Preparation of 2-(4-(benzylamino)phenyl)-5,7-difluoro-3-methylquinolin-4(1H)-one 17k. White solid (51%); m.p. 282-283°C. NMR: ¹H (400 MHz, DMSO) δ 11.39 (s, 1H), 7.36 (dt, *J* = 15.1, 7.4 Hz, 4H), 7.27 – 7.21 (m, 3H), 7.13 (d, *J* = 9.0 Hz, 1H), 6.97 (ddd, *J* = 12.0, 9.8, 2.3 Hz, 1H), 6.84 (t, *J* = 6.1 Hz, 1H), 6.72 (d, *J* = 8.6 Hz, 2H), 4.36 (d, *J* = 6.1 Hz, 2H), 1.86 (s, 3H); ¹³C (101 MHz, DMSO) δ 175.36, 164.04, 161.44, 150.01, 148.03, 142.58, 141.46, 140.25, 130.20, 128.73, 127.47, 127.09, 121.61, 116.08, 112.05, 99.34, 98.71, 46.39, 12.56. ES HRMS: *m/z* found 377.1465, C₂₃H₁₉N₂OF₂ requires 377.1465; Anal. C₂₃H₁₈N₂OF₂ requires C 73.39%, H 4.82%, N 7.44%, found C 73.18%, H 4.74%, N 7.41%.

Preparation of 2-(4-(dimethylamino)phenyl)-5,7-difluoro-3-methylquinolin-4(1H)-one 17l. White solid (46%); m.p. 294°C. NMR: ¹H (400 MHz, DMSO) δ 11.48 (s, 1H), 7.37 (d, *J* = 8.8 Hz,

2H), 7.17 (d, J = 10.1 Hz, 1H), 6.99 (ddd, J = 12.1, 9.6, 2.4 Hz, 1H), 6.86 (d, J = 8.9 Hz, 2H), 2.99 (s, 6H), 1.88 (s, 3H); ^{13}C (101 MHz, DMSO) δ 175.39, 164.08, 160.75, 151.31, 147.89, 142.77, 130.17, 121.70, 116.20, 111.89, 110.45, 99.38, 98.77, 40.24, 12.55. ES HRMS: m/z found 315.1319, $\text{C}_{18}\text{H}_{17}\text{N}_2\text{OF}_2$ requires 315.1309; Anal. $\text{C}_{18}\text{H}_{16}\text{N}_2\text{OF}_2$ requires C 68.78%, H 5.13%, N 8.91%, found C 68.47%, H 5.14%, N 8.78%.

Preparation of 2-(4-(4-benzylpiperidin-1-yl)phenyl)-3-methylquinolin-4(1H)-one 21a. White powder (Yield 33%); m.p 256-258 °C ^1H NMR (400MHz, DMSO), δ_{H} 11.39 (s, 1H, NH), 8.10 (d, 1H, J = 7.7 Hz, Ar), 7.63-7.55 (m, 2H, AR), 7.37 (d, 2H, J = 8.9 Hz, Ar), 7.33-7.24 (m, 3H, Ar), 7.22-7.17 (m, 3H, Ar), 7.06 (d, 2H, J = 8.9 Hz, Ar), 3.82 (d, 2H, J = 12.8 Hz, CH_2), 2.79-2.66 (m, 2H, CH_2), 2.56 (d, 2H, J = 7.0 Hz, CH_2Ar), 1.96 (s, 3H, CH_3), 1.79-1.73 (m, 1H, CH), 1.67 (d, 2H, J = 12.9 Hz, CH_2), 1.29 (qd, 2H, J = 12.6 Hz, 3.9 Hz, CH_2) ^{13}C NMR (100MHz, DMSO), δ_{C} 177.0, 151.9, 148.3, 140.5, 139.9, 131.3, 130.2, 129.4, 128.5, 126.2, 125.3, 124.5, 123.3, 122.7, 118.4, 115.0, 114.4, 48.7, 42.6, 37.7, 31.5, 12.8 MS (ES+), $[\text{M} + \text{H}]^+$ (100), 409.2, HRMS calculated for 409.2280 $\text{C}_{28}\text{H}_{29}\text{N}_2\text{O}$, found 409.2289; Anal. $\text{C}_{28}\text{H}_{28}\text{N}_2\text{O}$ requires C 82.32%, H 6.91%, N 6.86%, found C 81.98%, H 6.92%, N 6.88%.

Preparation of 2-(4-(4-benzylpiperidin-1-yl)phenyl)-6-fluoro-3-methylquinolin-4(1H)-one 21b. White powder (Yield 40%); m.p. 302-302 °C ^1H NMR (400MHz, DMSO), δ_{H} 11.55 (s, 1H, NH), 7.73 (dd, 1H, J = 9.5 Hz, 3.0 Hz, Ar), 7.68 (dd, 1H, J = 9.1 Hz, 4.7 Hz, Ar), 7.54-7.48 (m, 1H, Ar), 7.38 (d, 2H, J = 8.9 Hz, Ar), 7.33-7.27 (m, 2H, Ar), 7.23-7.17 (m, 3H, Ar), 7.06 (d, 2H, J = 8.9 Hz, Ar), 3.83 (d, 2H, J = 12.7 Hz, CH_2), 2.79-2.67 (m, 2H, CH_2), 2.55 (d, 2H, J = 7.0 Hz, CH_2Ar), 1.94 (s, 3H, CH_3), 1.80-1.68 (m, 1H, CH), 1.67 (d, 2H, J = 13.1 Hz, CH_2), 1.28 (qd, 2H, J = 12.6 Hz, 3.9 Hz, CH_2) ^{13}C NMR (100MHz, DMSO), δ_{C} 176.2, 157.1, 152.0, 148.6, 140.5, 136.6, 130.2, 129.4, 128.5, 126.2, 124.3, 121.2, 120.4, 115.0, 113.9, 109.1, 48.4, 42.6, 37.7, 31.4, 12.7 MS

(ES⁺), [M + H]⁺ (100), 427.2, HRMS calculated for 427.2186 C₂₈H₂₈N₂O₄F, found 427.2177; Anal. C₂₈H₂₇N₂O₄F requires C 78.85%, H 6.38%, N 6.57%, found C 78.31%, H 6.35%, N 6.63%.

Preparation of 2-(4-(4-benzylpiperidin-1-yl)phenyl)-7-methoxy-3-methylquinolin-4(1H)-one 21c.

Light yellow powder (Yield 42 %); m.p. 218-220 °C ¹H NMR (400MHz, DMSO), δ_H 11.21 (s, s, 1H, NH), 7.99 (d, 1H, J = 8.9 Hz, Ar), 7.36 (d, 2H, J = 8.7 Hz, Ar), 7.29 (d, 2H, J = 7.2 Hz, Ar), 7.20 (d, 3H, J = 6.4 Hz, Ar), 7.05 (d, 3H, J = 8.6 Hz, Ar), 6.87 (dd, 1H, J = 8.9 Hz, 2.4 Hz, Ar), 3.82 (s, 3H, OCH₃), 2.71 (t, 2H, J = 11.5 Hz, CH₂), 2.56 (d, 2H, J = 6.9 Hz, CH₂Ar), 1.91 (s, 3H, CH₃), 1.79-1.71 (m, 1H, CH), 1.29 (dt, 2H, J = 11.7 Hz, 8.9 Hz, CH₂) ¹³C NMR (100MHz, DMSO), δ_C 176.7, 161.8, 151.8, 147.8, 141.6, 140.5, 130.2, 129.4, 128.5, 127.1, 126.2, 124.6, 117.9, 115.0, 113.9, 113.0, 99.2, 55.6, 48.5, 42.6, 37.7, 31.5, 12.7 MS (ES⁺), [M + H]⁺ (100), 439.2 HRMS calculated for 439.2386 C₂₉H₃₁N₂O₂, found 439.2386; Purity HPLC 95% (Method B) R_t = 2.43 min.

Preparation of 2-(4-(4-benzylpiperazin-1-yl)phenyl)-3-methylquinolin-4(1H)-one 21d. White powder (Yield 30%); m.p. 258-260 °C ¹H NMR (400MHz, DMSO), δ_H 11.40 (s, 1H, NH), 8.10 (d, 1H, J = 7.7 Hz, AR), 7.63-7.55 (m, 2H, Ar), 7.40 (d, 2H, J = 8.9 Hz, Ar), 7.37-7.33 (m, 3H, Ar), 7.27 (ddd, 2H, J = 10.3 Hz, 5.5 Hz, 2.5 Hz, Ar), 7.08 (d, 2H, J = 8.9 Hz, Ar), 3.54 (s, 2H, CH₂Ar), 3.29-3.23 (m, 4H, NCH₂), 2.58-2.52 (m, 4H, CH₂N), 1.93 (s, 3H, CH₃) ¹³C NMR (100MHz, DMSO), δ_C 177.0, 151.9, 148.2, 139.9, 138.4, 131.4, 130.2, 129.3, 128.6, 127.4, 125.3, 123.3, 122.8, 118.4, 114.9, 114.4, 62.4, 52.8, 49.0, 48.1, 12.7 MS (ES⁺), [M + H]⁺ (100), 410.2, HRMS calculated for 410.2232 C₂₇H₂₈N₃O, found 410.2234; Anal. C₂₇H₂₇N₃O requires C 79.19%, H 6.65%, N 10.26%, found C 78.63%, H 6.66%, N 10.21%.

Preparation of 2-(4-(4-benzylpiperazin-1-yl)phenyl)-6-fluoro-3-methylquinolin-4(1H)-one 21e.

White powder (Yield 28%); m.p. 306-308 °C. ¹H NMR (400MHz, DMSO), δ_H 11.69 (s, 1H, NH),

7.74-7.71 (m, 2H, Ar), 7.54-7.48 (m, 1H, Ar), 7.40 (d, 2H, J = 8.9 Hz, Ar), 7.37-7.33 (m, 4H, Ar), 7.08 (d, 2H, J = 8.9 Hz, Ar), 3.54 (s, 2H, CH₂Ar), 3.30-3.22 (m, 4H, CH₂N), 2.59-2.52 (m, 4H, NCH₂), 1.94 (s, 3H, CH₃) ¹³C NMR (100MHz, DMSO), δ_C 151.9, 148.6, 138.4, 136.7, 130.2, 129.3, 128.6, 127.4, 124.9, 124.2, 120.4, 114.8, 113.9, 109.0, 62.4, 55.3, 52.8, 48.0, 12.8 MS (ES+), [M + H]⁺ (100), 428.2, HRMS calculated for 428.2138 C₂₇H₂₇N₃OF, found 428.2138; Purity HPLC 98% (Method A) R_t = 1.82 min..

*Preparation of 7-methoxy-3-methyl-2-(4-(4-phenylpiperazin-1-yl)phenyl)quinolin-4(1H)-one **21f**.*

White powder (Yield 30 %); m.p. 312-314 °C. ¹H NMR (400MHz, DMSO), δ_H 11.25 (s, 1H, NH), 8.01 (d, 1H, J = 9.0 Hz, Ar), 7.43 (d, 2H, J = 8.8 Hz, Ar), 7.26 (dd, 2H, J = 8.4 Hz, Ar), 7.16 (d, 2H, J = 8.8 Hz, Ar), 7.05 (d, 1H, J = 2.4 Hz, Ar), 7.02 (d, 2H, J = 8.0 Hz, Ar), 6.88 (dd, 1H, J = 9.0 Hz, Ar), 6.83 (t, 1H, J = 7.3 Hz, Ar), 3.82 (s, 3H, OCH₃), 3.41 (dd, 4H, J = 6.5 Hz, 3.5 Hz, NCH₂), 3.31 (dd, 4H, J = 6.5 Hz, 3.5 Hz, CH₂N), 1.92 (s, 3H, CH₃) ¹³C NMR (100MHz, DMSO), δ_C 176.7, 161.8, 151.7, 151.3, 147.7, 141.6, 130.2, 129.4, 127.1, 125.6, 119.6, 117.9, 116.1, 115.1, 114.0, 113.0, 99.2, 55.7, 48.6, 48.1, 12.6 MS (ES+), [M + H]⁺ (100), 426.2, HRMS calculated for 426.2182 C₂₇H₂₈N₃O₂, found 426.2184; Purity HPLC 91% (Method A) R_t = 1.80 min.

*Preparation of 2-(4-(4-benzylpiperazin-1-yl)phenyl)-7-methoxy-3-methylquinolin-4(1H)-one **21g**.*

White powder (Yield 38%); m.p. 280-282 °C. ¹H NMR (400MHz, DMSO), δ_H 11.22 (s, 1H, NH), 8.00 (d, 1H, J = 9.0 Hz, AR), 7.38 (d, 2H, J = 8.9 Hz, Ar), 7.37-7.33 (m, 4H, Ar), 7.31-7.24 (m, 1H, Ar), 7.07 (d, 2H, J = 8.9 Hz, Ar), 7.04 (d, 1H, J = 2.4 Hz, Ar), 6.87 (dd, 1H, J = 8.9 Hz, 2.4 Hz, Ar), 3.82 (s, 3H, OCH₃), 3.54 (s, 2H, NCH₂Ar), 3.29-3.23 (m, 4H, NCH₂), 2.57-2.52 (m, 4H, CH₂N), 1.91 (s, 3H, CH₃) ¹³C NMR (100MHz, DMSO), δ_C 176.7, 161.8, 151.8, 147.7, 141.6, 138.4, 130.1, 129.3, 128.6, 127.4, 125.3, 117.9, 114.8, 113.0, 99.2, 62.4, 55.6, 52.8, 49.0, 48.0, 12.6 MS (ES+), [M + H]⁺ (100), 440.2, HRMS calculated for 440.2338 C₂₈H₃₀N₃O₂, found

440.2344; Anal. C₂₈H₂₉N₃O₂ requires C 76.51%, H 6.65%, N 9.56%, found C 76.12%, H 6.63%, N 9.48%.

Preparation of 5,7-difluoro-3-methyl-2-(3-(piperidin-1-yl)phenyl)quinolin-4(1H)-one 24. White solid (Yield 45%); m.p. 269 – 270°C. ¹H NMR (400 MHz, DMSO) δ_H 11.63 (s, 1H, NH), 7.37 (t, *J* = 7.9 Hz, 1H, Ar), 7.16 (d, *J* = 9.8 Hz, 1H, Ar), 7.10 (dd, *J* = 8.4, 2.3 Hz, 1H, Ar), 7.06 – 6.97 (m, 2H, Ar), 6.86 (d, *J* = 7.5 Hz, 1H, Ar), 3.27 – 3.19 (m, 4H, CH₂), 1.83 (s, 3H, CH₃), 1.62 (d, *J* = 4.0 Hz, 4H, CH₂), 1.59 – 1.50 (m, 2H, CH₂); ¹³C NMR (101 MHz, DMSO) δ 175.48, 163.86 (dd, *J* = 65.8, 15.2 Hz), 161.33 (dd, *J* = 80.6, 14.7 Hz), 151.93, 148.14, 142.72 (dd, *J* = 14.7, 6.4 Hz), 135.62, 129.68, 118.86, 116.90, 116.66, 116.07, 110.74 (d, *J* = 10.7 Hz), 99.66 (dd, *J* = 24.4, 4.5 Hz), 99.06 (dd, *J* = 26.8, 25.8 Hz), 49.65, 25.57, 24.33, 12.44. HRMS (ESI) C₂₁H₂₀N₂OF₂²³Na [M+H]⁺ requires 377.1441, found 377.1448 (100%). Anal. C₂₁H₂₀N₂OF₂ requires C 71.17%, H 5.69%, N 7.90%, found C 70.78%, H 5.59%, N 7.64%.

Preparation of 1-(4-(5,7-difluoro-3-methyl-4-oxo-1,4-dihydroquinolin-2-yl)phenyl)-1H-pyrrole-2-carbonitrile 32a. White solid (55%); m.p. 312°C. NMR: ¹H (400 MHz, DMSO) δ 11.81 (s, 1H), 7.84 – 7.74 (m, 4H), 7.67 (dd, *J* = 2.8, 1.6 Hz, 1H), 7.31 (dd, *J* = 4.0, 1.6 Hz, 1H), 7.15 (d, *J* = 10.0 Hz, 1H), 7.06 (ddd, *J* = 12.0, 9.6, 2.4 Hz, 1H), 6.52 (dd, *J* = 3.9, 2.8 Hz, 1H), 1.86 (s, 3H); ¹³C (101 MHz, DMSO) δ 175.33, 161.19, 146.24, 142.77, 138.87, 134.57, 130.86, 128.96, 128.22, 124.59, 123.61, 117.07, 114.20, 111.64, 110.81, 103.09, 99.47, 99.21, 12.25; HRMS (ESI) C₂₁H₁₄N₃OF₂ [M+H]⁺ requires 362.1099, found 362.1108 (100%). Anal. C₂₁H₁₃N₃OF₂ requires C 69.80%, H 3.63%, N 11.63%, found C 69.67%, H 3.66%, N 11.38%.

Preparation of 2-(4-(1H-indol-1-yl)phenyl)-5,7-difluoro-3-methylquinolin-4(1H)-one 32b. while solid (57%); m.p. decomposed at 325°C. NMR: ¹H (400 MHz, DMSO) δ 11.79 (s, 1H), 7.84 (d, *J* = 8.5 Hz, 2H), 7.81 – 7.74 (m, 3H), 7.69 (t, *J* = 8.6 Hz, 2H), 7.26 (t, *J* = 7.7 Hz, 1H), 7.22 – 7.14

(m, 2H), 7.06 (ddd, $J = 12.0, 9.7, 2.4$ Hz, 1H), 6.78 (d, $J = 3.3$ Hz, 1H), 1.91 (s, 3H); ^{13}C (101 MHz, DMSO) δ 175.38, 146.64, 142.71, 140.41, 137.96, 135.25, 132.43, 130.93, 129.74, 128.77, 123.87, 122.96, 121.51, 120.98, 117.40, 117.01, 110.75, 104.64, 99.47, 99.13, 96.43, 12.35; HRMS (ESI) $\text{C}_{24}\text{H}_{17}\text{N}_2\text{OF}_2$ $[\text{M}+\text{H}]^+$ requires 387.1303, found 387.1300 (100%). Anal. $\text{C}_{24}\text{H}_{16}\text{N}_2\text{OF}_2$ requires C 74.60%, H 4.17%, N 7.25%, found C 74.21%, H 4.17%, N 7.24%.

Preparation of 2-(4-(1H-pyrazol-1-yl)phenyl)-5,7-difluoro-3-methylquinolin-4(1H)-one 32c.

White solid (Yield 35%); m.p. 306°C. ^1H NMR (400 MHz, DMSO) δ_{H} 11.73 (s, 1H, NH), 8.66 (d, $J = 2.5$ Hz, 1H), 8.07 (d, $J = 8.6$ Hz, 2H), 7.83 (d, $J = 1.6$ Hz, 1H), 7.70 (d, $J = 8.5$ Hz, 2H), 7.16 (d, $J = 9.8$ Hz, 1H), 7.05 (ddd, $J = 11.9, 9.7, 2.3$ Hz, 1H), 6.74 – 6.53 (m, 1H), 1.87 (s, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO) δ 175.47 (C=O), 163.94 (dd, $J = 72.7, 14.9$ Hz, C-F), 161.40 (dd, $J = 87.4, 15.3$ Hz, C-F), 146.70, 142.81 (dd, $J = 14.6, 6.2$ Hz), 142.04, 140.83, 132.36, 130.81, 128.55, 118.62, 117.04, 110.80 (d, $J = 8.8$ Hz), 108.85, 99.70 (dd, $J = 24.4, 4.5$ Hz), 99.09 (d, $J = 25.2$ Hz), 12.40 (CH_3); HRMS (ESI) $\text{C}_{19}\text{H}_{13}\text{N}_3\text{OF}_2^{23}\text{Na}$ $[\text{M}+\text{Na}]^+$ requires 360.0924, found 360.0935. Anal. $\text{C}_{19}\text{H}_{13}\text{N}_3\text{OF}_2$ requires C 67.65%, H 3.88%, N 12.46%, found C 67.26%, H 4.00%, N 12.24%.

Preparation of 2-(4-(1H-pyrrol-1-yl)phenyl)-5-fluoro-3-methylquinolin-4(1H)-one 32d.

White solid (Yield 32%); m.p. >300°C. ^1H NMR (400 MHz, DMSO) δ_{H} 11.66 (s, 1H, NH), 7.96 – 7.73 (m, 2H), 7.67 – 7.61 (m, 2H), 7.61 – 7.49 (m, 3H), 7.42 (d, $J = 8.4$ Hz, 1H), 6.97 (dd, $J = 12.1, 7.9$ Hz, 1H), 6.49 – 6.14 (m, 2H), 1.88 (s, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO) δ_{C} 175.85 (C=O), 162.15, 159.57, 146.57, 142.23, 142.21 (d, $J = 4.4$ Hz), 140.89, 132.06 (d, $J = 10.8$ Hz), 131.60, 130.84, 119.35 (d, $J = 12.6$ Hz), 116.59, 114.53, 113.36 (d, $J = 8.8$ Hz), 111.37, 108.68 (d, $J = 20.9$ Hz), 12.46 (CH_3). HRMS (ESI) $\text{C}_{20}\text{H}_{15}\text{N}_2\text{OF}^{23}\text{Na}$ $[\text{M}+\text{Na}]^+$ requires 341.1066, found

341.1080. Anal. C₂₀H₁₅N₂OF requires C 75.46%, H 4.75%, N 8.80%, found C 75.23%, H 4.70%, N 8.72%.

Preparation of 2-(4-(3,4-difluoro-1H-pyrrol-1-yl)phenyl)-5,7-difluoro-3-methylquinolin-4(1H)-one 32e. White solid (38 mgs, 30 %). ¹H NMR (400 MHz, DMSO) 11.78 (bs, 1H), 7.83 (d, *J* = 8.8 Hz, 2H), 7.72 (d, *J* = 8.8 Hz, 4H), 7.14 (d, *J* = 9.6 Hz, 1H), 7.11 (dd, *J* = 11.0, 10.6 Hz, 1H), 1.91 (s, 3H), ¹³C NMR (100 MHz, DMSO) δ_{C} 175.1, 146.8, 140.3, 131.8, 130.8, 118.7, 116.9, 103.0, 12.3; MS (ES⁺) *m/z* 373 (M + H)⁺ HRMS calculated for 373.0964 C₂₀H₁₃N₂OF₄, found 373.0965; Purity HPLC 98% (Method A) R_t = 2.29 min.

Preparation of 2-(3-chloro-4-(1H-pyrrol-1-yl)phenyl)-5,7-difluoro-3-methylquinolin-4(1H)-one 32f. White solid (Yield 39%); m.p. 297 – 298°C. ¹H NMR (400 MHz, DMSO) δ_{H} 11.79 (s, 1H, NH), 7.92 (s, 1H), 7.75 – 7.57 (m, 2H), 7.19 – 7.01 (m, 4H), 6.32 (t, *J* = 2.1 Hz, 2H), 1.87 (s, 3H, CH₃); ¹³C NMR (101 MHz, DMSO) δ 175.42, 164.30, 161.92 (d, *J* = 14.8 Hz), 160.97 (d, *J* = 15.3 Hz), 145.27, 142.84, 139.25, 134.90, 131.52, 129.60, 128.42, 128.35, 122.63, 117.30, 110.23, 99.72 (d, *J* = 19.1 Hz), 99.24 (d, *J* = 26.0 Hz), 12.30; HRMS (ESI) C₂₀H₁₃N₂OF₂³⁵Cl²³Na [M+Na]⁺ requires 393.0582, found 393.0592. Anal. C₂₀H₁₃N₂OF requires C 64.79%, H 3.53%, N 7.56%, found C 64.66%, H 3.69%, N 7.39%.

Preparation of 5,7-difluoro-2-(2-fluoro-4-(1H-pyrrol-1-yl)phenyl)-3-methylquinolin-4(1H)-one 32g. White solid (Yield 39%); m.p. 307°C. ¹H NMR (400 MHz, DMSO) δ_{H} 11.82 (s, 1H, NH), 7.84 (dd, *J* = 11.8, 1.9 Hz, 1H), 7.77 – 7.64 (m, 2H), 7.62 – 7.55 (m, 2H), 7.18 – 6.99 (m, 2H), 6.40 – 6.23 (m, 2H), 1.79 (s, 3H, CH₃); HRMS (ESI) C₂₀H₁₄N₂OF₃ [M+H]⁺ requires 355.1058, found 355.1074. Anal. C₂₀H₁₃N₂OF₃ requires C 67.79%, H 3.70%, N 7.91%, found C 66.94%, H 3.68%, N 7.73%.

Preparation of (R)-5,7-difluoro-2-(4-(3-fluoropyrrolidin-1-yl)phenyl)-3-methylquinolin-4(1H)-one 38a. White solid (45%); m.p. 313-314°C. NMR: ¹H (400 MHz, DMSO) δ 11.47 (s, 1H), 7.38 (d, J = 8.7 Hz, 2H), 7.18 (d, J = 9.3 Hz, 1H), 6.99 (ddd, J = 12.0, 9.6, 2.4 Hz, 1H), 6.73 (d, J = 8.7 Hz, 2H), 5.50 (d, J = 54.1 Hz, 1H), 3.71 – 3.36 (m, 4H), 2.38 – 2.12 (m, 2H), 1.89 (s, 3H); ¹³C (101 MHz, DMSO) δ 175.38, 148.31, 147.92, 142.70, 130.35, 121.60, 116.19, 111.70, 110.56, 99.39, 98.76, 94.49, 92.78, 54.48, 45.59, 32.14, 31.93, 12.58. ES HRMS: m/z found 359.1385, C₂₀H₁₈N₂OF₃ requires 359.1371; Anal. C₂₀H₁₇N₂OF₃ requires C 67.03%, H 4.78%, N 7.82%, found C 67.26%, H 4.73%, N 7.81%.

Preparation of (S)-5,7-difluoro-2-(4-(3-fluoropyrrolidin-1-yl)phenyl)-3-methylquinolin-4(1H)-one 38b. White solid (47%); m.p. 313-314°C. NMR: ¹H (400 MHz, DMSO) δ 11.47 (s, 1H), 7.38 (d, J = 8.6 Hz, 2H), 7.18 (d, J = 9.2 Hz, 1H), 6.99 (ddd, J = 12.0, 9.7, 2.4 Hz, 1H), 6.73 (d, J = 8.7 Hz, 2H), 5.50 (d, J = 54.3 Hz, 1H), 3.69 – 3.36 (m, 4H), 2.36 – 2.13 (m, 2H), 1.89 (s, 3H); ¹³C (101 MHz, DMSO) δ 175.38, 148.32, 147.93, 142.78, 130.36, 121.60, 116.19, 111.70, 110.54, 99.36, 98.76, 94.49, 92.78, 54.48, 45.59, 32.14, 31.93, 12.58. ES HRMS: m/z found 359.1381, C₂₀H₁₈N₂OF₃ requires 359.1371; Anal. C₂₀H₁₇N₂OF₃ requires C 67.03%, H 4.78%, N 7.82%, found C 67.25%, H 4.67%, N 7.86%.

Preparation of 2-(4-(3,3-difluoroazetidin-1-yl)phenyl)-5,7-difluoro-3-methylquinolin-4(1H)-one 38c. White solid (33%); m.p. 316-318°C. NMR: ¹H (400 MHz, DMSO) δ 11.54 (s, 1H), 7.43 (d, J = 8.4 Hz, 2H), 7.16 (d, J = 9.6 Hz, 1H), 7.01 (t, J = 10.8 Hz, 1H), 6.74 (d, J = 8.5 Hz, 2H), 4.37 (t, J = 12.3 Hz, 4H), 1.86 (s, 3H).; ¹³C (101 MHz, DMSO) δ 175.39, 150.88, 147.53, 142.81, 130.18, 124.67, 117.01, 116.50, 112.70, 110.53, 99.41, 98.90, 90.56, 74.81, 63.29, 12.44. ES HRMS: m/z found 363.1130, C₁₉H₁₅N₂OF₄ requires 363.1121; Anal. C₁₉H₁₄N₂OF₄ requires C 62.98%, H 3.89%, N 7.73%, found C 63.03%, H 3.79%, N 7.71%.

Preparation of 2-(4-(3,4-difluoro-1H-pyrrol-1-yl)phenyl)-5,7-difluoro-3-methylquinolin-4(1H)-one 38d. White solid (38 mgs, 30 %). ¹H NMR (400 MHz, DMSO) 11.78 (bs, 1H), 7.83 (d, *J* = 8.8 Hz, 2H), 7.72 (d, *J* = 8.8 Hz, 4H), 7.14 (d, *J* = 9.6 Hz, 1H), 7.11 (dd, *J* = 11.0, 10.6 Hz, 1H), 1.91 (s, 3H); ¹³C NMR (100 MHz, DMSO) δ_C 175.1, 146.8, 140.3, 131.8, 130.8, 118.7, 116.9, 103.0, 12.3; MS (ES⁺) *m/z* 373 (M + H)⁺ HRMS calculated for 373.0964 C₂₀H₁₃N₂OF₄, found 373.0965; Purity HPLC 98% (Method A) R_t = 2.60 min..

Preparation of 2-(4-(3,4-difluoro-1H-pyrrol-1-yl)phenyl)-7-methoxy-3-methylquinolin-4(1H)-one 38e. White solid (0.12 g, 32 %). ¹H NMR (400 MHz, DMSO) 11.48 (bs, 1H), 8.02 (d, *J* = 9.2 Hz, 1H), 7.75 (d, *J* = 8.8 Hz, 2H), 7.66 (m, 4H), 7.01 (s, 1H), 6.90 (d, *J* = 9.0 Hz, 1H), 3.82 (s, 3H), 1.90 (s, 3H); ¹³C NMR (100 MHz, DMSO) δ_C 176.5, 161.9, 141.8, 141.1, 140.0, 138.9, 138.7, 130.8, 127.2, 118.6, 118.0, 114.3, 113.3, 102.7, 102.5, 102.4, 99.2, 55.7, 12.5; MS (ES⁺) *m/z* 367 (M + H)⁺ HRMS calculated for 367.1258 C₂₁H₁₇N₂O₂F₂, found 367.1257; Purity HPLC 99+% (Method A) R_t = 2.09 min.

Preparation of 6-chloro-2-(4-(3,4-difluoro-1H-pyrrol-1-yl)phenyl)-7-methoxy-3-methylquinolin-4(1H)-one 38f. White solid (0.11 g, 30 %). ¹H NMR (400 MHz, DMSO) 11.75 (bs, 1H), 8.03 (s, 1H), 7.71 (d, *J* = 8.8 Hz, 2H), 7.63 (m, 4H), 7.15 (s, 1H), 3.89 (s, 3H), 1.91 (s, 3H); ¹³C NMR (100 MHz, DMSO) δ_C 175.1, 156.3, 139.7, 138.7, 130.8, 126.0, 118.5, 114.2, 102.7, 102.5, 102.4, 56.5, 12.9; MS (ES⁺) *m/z* 401 (M + H)⁺ HRMS calculated for 401.0868 C₂₁H₁₆N₂O₂F₂³⁵Cl, found 401.0870; Purity HPLC 97% (Method A) R_t = 2.35 min..

Preparation of 5,7-difluoro-2-(4-(3-hydroxy-3-methylpiperidin-1-yl)phenyl)-3-methylquinolin-4(1H)-one 38g. White solid (48%); m.p. decomposed at 284°C. NMR: ¹H (400 MHz, DMSO) δ 11.47 (s, 1H), 7.35 (d, *J* = 8.8 Hz, 2H), 7.16 (d, *J* = 9.2 Hz, 1H), 7.07 – 6.94 (m, 3H), 4.46 (s, 1H), 3.30 – 3.02 (m, 4H), 1.88 (s, 3H), 1.86 – 1.75 (m, 1H), 1.63 – 1.48 (m, 3H), 1.17 (s, 3H); ¹³C (101

MHz, DMSO) δ 175.37, 163.50, 161.49, 152.33, 147.68, 142.79, 130.15, 123.19, 116.27, 114.64, 110.56, 99.34, 98.82, 67.64, 59.76, 47.81, 37.73, 27.28, 22.10, 12.52. ES HRMS: m/z found 385.1738, $C_{22}H_{23}N_2O_2F_2$ requires 385.1728; Anal. $C_{22}H_{22}N_2O_2F_2$ requires C 68.74%, H 5.77%, N 7.29%, found C 68.49%, H 5.84%, N 7.39%.

Preparation of 5,7-difluoro-2-(4-(3-hydroxy-3-methylpyrrolidin-1-yl)phenyl)-3-methylquinolin-4(1H)-one 38h. White solid (50%); m.p. 288-290°C. NMR: 1H (400 MHz, DMSO) δ 11.43 (s, 1H), 7.35 (d, J = 8.7 Hz, 2H), 7.18 (d, J = 10.1 Hz, 1H), 6.98 (ddd, J = 12.0, 9.6, 2.5 Hz, 1H), 6.62 (d, J = 8.8 Hz, 2H), 4.85 (s, 1H), 3.48 – 3.36 (m, 2H), 3.24 (s, 2H), 2.01 – 1.92 (m, 2H), 1.89 (s, 3H), 1.37 (s, 3H); ^{13}C (101 MHz, DMSO) δ 175.38, 160.89, 155.31, 148.73, 148.07, 130.29, 120.65, 116.06, 111.02, 99.38, 96.34, 94.24, 91.71, 75.74, 60.95, 55.28, 46.88, 26.29, 12.63. ES HRMS: m/z found 399.1391, $C_{21}H_{20}N_2O_2F_2^{23}Na$ requires 393.1391; Purity HPLC 98% (Method A) R_t = 2.25 min.

Preparation of 5,7-difluoro-2-(4-(3-hydroxy-3-methylazetidin-1-yl)phenyl)-3-methylquinolin-4(1H)-one 38i. White solid (43%); m.p. decomposed at 289°C. NMR: 1H (400 MHz, DMSO) δ 11.48 (s, 1H), 7.35 (d, J = 8.6 Hz, 2H), 7.16 (d, J = 9.1 Hz, 1H), 6.99 (ddd, J = 12.0, 9.6, 2.4 Hz, 1H), 6.58 (d, J = 8.6 Hz, 2H), 5.60 (s, 1H), 3.83 (d, J = 7.9 Hz, 2H), 3.69 (d, J = 7.7 Hz, 2H), 1.86 (s, 3H), 1.48 (s, 3H); ^{13}C (101 MHz, DMSO) δ 175.38, 160.90, 152.74, 147.89, 142.69, 136.81, 134.24, 130.07, 122.72, 116.28, 111.45, 99.62, 98.81, 67.73, 66.17, 27.02, 12.52. ES HRMS: m/z found 379.1237, $C_{20}H_{18}N_2O_2F_2^{23}Na$ requires 379.1234; Anal. $C_{20}H_{18}N_2O_2F_2$ requires C 67.41%, H 5.09%, N 7.86%, found C 67.18%, H 5.49%, N 7.24%.

Preparation of (S)-2-(4-(2-((benzyloxy)methyl)pyrrolidin-1-yl)phenyl)-5,7-difluoro-3-methylquinolin-4(1H)-one 38j. Cream solid (0.10 g, 20 %). 1H NMR (400 MHz, DMSO) 10.60 (bs, 1H), 7.33 (m, 6H), 7.22 (d, J = 8.8 Hz, 2H), 6.56 (dd, J = 11.0, 10.6 Hz, 1H), 6.44 (d, J = 8.8

Hz, 2H), 4.52 (s, 2H), 3.84 (m, 1H), 3.51 (dd, $J = 8.8, 4.5$ Hz, 1H), 3.30 (m, 2H), 3.05 (m, 1H), 2.05 (m, 4H), 1.92 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ_{C} 177.1, 148.8, 147.9, 138.1, 129.7, 128.4, 127.8, 127.6, 121.5, 117.2, 111.3, 99.2, 73.4, 70.0, 58.2, 48.3, 28.9, 23.2, 12.4; MS (ES^+) m/z 461 ($\text{M} + \text{H}$) $^+$ HRMS calculated for 461.2041 $\text{C}_{28}\text{H}_{27}\text{N}_2\text{O}_2\text{F}_2$, found 461.2055.

General procedure for the preparation of compounds 39a-c.

Preparation of (S)-5,7-difluoro-2-(4-(2-(hydroxymethyl)pyrrolidin-1-yl)phenyl)-3-methylquinolin-4(1H)-one 39a. Cream solid (50 mgs, 90 %). ^1H NMR (400 MHz, DMSO) 11.45 (bs, 1H), 7.35 (d, $J = 8.8$ Hz, 2H), 7.20 (dd, $J = 8.0, 4.5$ Hz, 1H), 7.01 (dd, $J = 11.0, 10.6$ Hz, 1H), 6.75 (d, $J = 8.8$ Hz, 2H), 4.90 (m, 1H), 3.81 (m, 1H), 3.75 (m, 1H), 3.50 (m, 1H), 3.22 (m, 1H), 3.10 (m, 1H), 2.03 (m, 4H), 1.92 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ_{C} 175.4, 148.4, 148.0, 130.3, 121.1, 116.1, 111.7, 99.3, 61.3, 60.5, 48.5, 28.3, 23.0, 12.6; MS (ES^+) m/z 371 ($\text{M} + \text{H}$) $^+$ HRMS calculated for 371.1571 $\text{C}_{21}\text{H}_{21}\text{N}_2\text{O}_2\text{F}_2$, found 371.1568; Purity HPLC 96% (Method A) $R_t = 2.25$ min.

Preparation of (R)-5,7-difluoro-2-(4-(2-(hydroxymethyl)pyrrolidin-1-yl)phenyl)-3-methylquinolin-4(1H)-one 39b. Light yellow solid (0.065 g, 85 %). ^1H NMR (400 MHz, DMSO) 11.44 (bs, 1H), 7.35 (d, $J = 8.8$ Hz, 2H), 7.17 (d, $J = 8.0$ Hz, 1H), 6.99 (dd, $J = 11.0, 10.6$ Hz, 1H), 6.75 (d, $J = 8.8$ Hz, 2H), 4.84 (dd, $J = 5.8, 5.8$ Hz, 1H), 3.77 (m, 1H), 3.51 (m, 1H), 3.42 (m, 1H), 3.25 (m, 1H), 3.08 (m, 1H), 1.98 (m, 4H), 1.89 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ_{C} 175.7, 148.8, 148.0, 130.3, 121.1, 116.2, 111.7, 99.9, 61.5, 60.5, 28.5, 23.6, 12.6; MS (ES^+) m/z 371 ($\text{M} + \text{H}$) $^+$ HRMS calculated for 371.1571 $\text{C}_{21}\text{H}_{21}\text{N}_2\text{O}_2\text{F}_2$, found 371.1572; Purity HPLC 97% (Method A) $R_t = 2.24$ min.

Preparation of (R)-2-(4-(3-(aminomethyl)pyrrolidin-1-yl)phenyl)-5,7-difluoro-3-methylquinolin-4(1H)-one 39c. White solid (21 mgs, 93 %). ^1H NMR (400 MHz, DMSO) 11.48 (bs, 1H), 7.40 (m,

1H), 7.38 (d, $J = 8.8$ Hz, 2H), 7.21 (d, $J = 8.4$ Hz, 1H), 6.99 (dd, $J = 11.0, 10.6$ Hz, 1H), 6.86 (d, $J = 8.8$ Hz, 2H), 4.09 (m, 1H), 3.20 (m, 1H), 2.99 (m, 1H), 2.51 (d, $J = 10.4$ Hz, 1H), 2.31 (dd, $J = 14.4, 10.9$ Hz, 1H), 2.13 (m, 1H), 1.98 (s, 3H), 1.82 (m, 2H), 1.63 (m, 2H); ^{13}C NMR (100 MHz, DMSO) δ_{C} 175.4, 147.8, 130.4, 116.2, 112.0, 111.6, 99.7, 56.5, 56.2, 48.3, 34.6, 28.5, 12.6; MS (ES^+) m/z 370 ($\text{M} + \text{H}$) $^+$ HRMS calculated for 370.1731 $\text{C}_{21}\text{H}_{22}\text{N}_3\text{OF}_2$, found 370.1738; Purity HPLC 96% (Method A) $R_t = 1.61$ min.

Preparation of 2-(4-(3,3-difluoropyrrolidin-1-yl)phenyl)-5,7-difluoro-3-methylquinolin-4(1H)-one 42a. White solid (56%); m.p. decomposed at 316°C. NMR: ^1H (400 MHz, DMSO) δ 7.41 (d, $J = 8.7$ Hz, 2H), 7.17 (d, $J = 9.0$ Hz, 1H), 7.01 (ddd, $J = 12.0, 9.6, 2.4$ Hz, 1H), 6.78 (d, $J = 8.8$ Hz, 2H), 3.79 (t, $J = 13.3$ Hz, 1H), 3.56 (t, $J = 7.2$ Hz, 1H), 2.59 (tt, $J = 14.5, 7.3$ Hz, 1H), 1.87 (s, 1H); ^{13}C (101 MHz, DMSO) δ 175.38, 164.09, 148.09, 147.72, 142.82, 130.34, 129.16, 126.71, 122.79, 116.32, 111.98, 111.61, 99.37, 98.82, 54.96, 45.75, 33.72, 12.54. ES HRMS: m/z found 399.1093, $\text{C}_{20}\text{H}_{16}\text{N}_2\text{OF}_4^{23}\text{Na}$ requires 399.1096; Anal. $\text{C}_{20}\text{H}_{16}\text{N}_2\text{OF}_4$ requires C 63.83%, H 4.29%, N 7.44%, found C 63.49%, H 4.31%, N 7.28%.

Preparation of 2-(4-(3,3-difluoropiperidin-1-yl)phenyl)-5,7-difluoro-3-methylquinolin-4(1H)-one 42b. White solid (47%); m.p. decomposed at 297°C. NMR: ^1H (400 MHz, DMSO) δ 11.54 (s, 1H), 7.39 (d, $J = 8.8$ Hz, 2H), 7.20 – 7.11 (m, 3H), 7.01 (ddd, $J = 12.0, 9.6, 2.4$ Hz, 1H), 3.65 (t, $J = 11.9$ Hz, 2H), 3.43 – 3.37 (m, 2H), 2.16 – 2.01 (m, 2H), 1.87 (s, 3H), 1.85 – 1.75 (m, 2H); ^{13}C (101 MHz, DMSO) δ 175.38, 152.75, 150.88, 147.47, 142.69, 130.26, 124.51, 121.44, 116.43, 115.09, 113.88, 110.51, 99.67, 98.87, 53.21, 52.92, 46.93, 32.09, 21.59, 12.47. ES HRMS: m/z found 391.1441, $\text{C}_{21}\text{H}_{19}\text{N}_2\text{OF}_4$ requires 391.1434; Anal. $\text{C}_{21}\text{H}_{18}\text{N}_2\text{OF}_4$ requires C 64.61%, H 4.65%, N 7.18%, found C 64.06%, H 4.61%, N 7.05%.

Preparation of (R)-N-(tert-butyl)-1-(4-(3-methyl-4-oxo-1,4-dihydroquinolin-2-yl)phenyl)pyrrolidine-2-carboxamide 45a. Pale yellow powder (yield 20%); m.p. 164-166 °C ¹H NMR (400 MHz, CDCl₃-d₆) δ_H 11.11 (s, 1H, NH), 7.43 (d, 2H, J = 8.6 Hz, Ar), 7.34 (d, 1H, J = 9.6 Hz, Ar), 6.71-6.61 (m, 1H, Ar), 6.55 (d, 2H, J = 8.6 Hz, Ar), 6.28 (s, 1H, NH), 3.59 (t, 1H, J = 7.2 Hz, CH), 2.99 (dd, 1H, J = 15.4 Hz, 8.9 Hz, CH₂), 2.89 (d, 1H, J = 8.6 Hz, CH₂), 2.03 (s, 3H, CH₃), 1.92-1.65 (m, 4H, CH₂), 1.34 (m, 9H, CH₃) ¹³C NMR (100 MHz, CDCl₃-d₆) δ_c 173.1, 148.0, 130.1, 125.3, 117.7, 113.1, 64.6, 51.3, 49.8, 31.4, 28.6, 24.0, 12.3 MS (ES⁺), [M + Na]⁺ (100) 462.2 HRMS calculated for 462.1969 C₂₅H₂₇O₂N₃F₂Na, found 462.1955; Anal. C₂₅H₂₇N₃O₂F₂ requires C 68.32%, H 6.19%, N 9.56%, found C 68.13%, H 6.10%, N 9.11%.

Preparation of (R)-1-(4-(5,7-difluoro-3-methyl-4-oxo-1,4-dihydroquinolin-2-yl)phenyl)-N,N-dimethylpyrrolidine-2-carboxamide 45b. Pale yellow powder (Yield 34%); m.p. 176-178 °C. ¹H NMR (400 MHz, CDCl₃-d₆) δ_H 10.40 (s, 1H, NH), 7.24-7.22 (m, 1H, Ar), 7.12 (d, 2H, J = 8.6 Hz, Ar), 6.73-6.56 (m, 1H, Ar), 6.13 (d, 2H, J = 8.6 Hz, Ar), 4.22 (dd, 1H, J = 8.8 Hz, 2.1 Hz, CH), 3.46-3.39 (m, 1H, CH₂), 3.25 (dd, 1H, J = 16.0 Hz, 8.4 Hz, CH₂), 3.16 (s, 3H, NCH₃), 2.85 (s, 3H, NCH₃), 2.35-2.23 (m, 1H, CH₂), 2.20-1.95 (m, 3H, CH₂), 1.90 (s, 3H, CH₃) ¹³C NMR (100 MHz, CDCl₃-d₆) δ_c 177.7, 172.7, 147.9, 129.6, 122.1, 117.1, 111.0, 58.6, 48.5, 36.9, 36.0, 30.5, 23.6, 15.3, 12.5 MS (ES⁺), [M + Na]⁺ (100) 434.2 HRMS calculated for 434.1656 C₂₃H₂₃O₂N₃F₂Na, found 434.1669; Purity HPLC 97% (Method B) R_t = 1.95 min.

Preparation of (R)-1-(4-(5,7-difluoro-3-methyl-4-oxo-1,4-dihydroquinolin-2-yl)phenyl)-N-(tetrahydro-2H-pyran-4-yl)pyrrolidine-2-carboxamide 45c. Pale yellow powder (yield 25%) m.p. 228-230 °C ¹H NMR (400 MHz, CDCl₃-d₆) δ_H 10.82 (s, 1H, NH), 7.36 (d, 2H, J = 8.7 Hz, Ar), 7.25 (d, 1H, J = 9.6 Hz, Ar), 6.68-6.59 (m, 1H, Ar), 6.56 (s, 1H, NH), 6.53 (d, 2H, J = 8.7 Hz, Ar), 4.06-3.82 (m, 2H, CH/CH₂), 3.66-3.56 (m, 1H, CH₂), 3.52-3.39 (m, 3H, CH₂), 3.30 (d, 1H, J = 6.7

Hz, CH₂), 3.11-3.02 (m, 1H, CH₂), 2.05-1.71 (m, 9H, CH₂/CH₃), 1.53-1.30 (m, 2H, CH₂) ¹³C NMR (100 MHz, CDCl₃-d₆) δ_c 177.1, 173.1, 148.0, 147.2, 130.0, 124.9, 117.6, 112.9, 66.6, 65.9, 64.1, 49.7, 46.0, 32.9, 31.4, 24.1, 15.3, 12.3 MS (ES+), [M + Na]⁺ (100) 490.2 HRMS calculated for 490.1018 C₂₆H₂₇O₃N₃F₂Na, found 490.1932; Purity HPLC 93% (Method B) R_t = 1.92 min.

Preparation of (R)-5,7-difluoro-3-methyl-2-(4-(2-(morpholine-4-carbonyl)pyrrolidin-1-yl)phenyl)quinolin-4(1H)-one 45d. Pale yellow powder (yield 18%); m.p. 236-238 °C. ¹H NMR (400 MHz, CDCl₃-d₆) δ_H 10.26 (s, 1H, NH), 7.20 (d, 1H, J = 9.2 Hz, Ar), 7.14 (d, 2H, J = 8.6 Hz, Ar), 6.67-6.59 (m, 1H, Ar), 6.17 (d, 2H, J = 8.6 Hz, Ar), 4.44-4.37 (m, 1H, CH), 3.78 (dd, 1H, CH₂), 3.74-3.55 (m, 6H, CH₂), 3.46-3.35 (m, 2H, CH₂), 3.27 (dd, 1H, J = 16.1 Hz, 8.3 Hz, CH₂), 2.36-2.24 (m, 1H, CH₂), 2.18-2.04 (m, 2H, CH₂), 2.03-1.96 (m, 1H, CH₂), 1.90 (s, 3H, CH₃) ¹³C NMR (100 MHz, CDCl₃-d₆) δ_c 171.2, 147.7, 147.0, 129.6, 122.2, 117.2, 111.1, 67.0, 66.5, 58.7, 48.5, 45.8, 42.5, 30.8, 23.6, 12.4 MS (ES+), [M + Na]⁺ (100) 476.2 HRMS calculated for 476.1762 C₂₅H₂₅O₃N₃F₂Na, found 476.1778; Purity HPLC 93% (Method B) R_t = 1.90 min.

Preparation of (R)-5,7-difluoro-2-(4-(2-(4-fluoropiperidine-1-carbonyl)pyrrolidin-1-yl)phenyl)-3-methylquinolin-4(1H)-one 45e. Pale yellow powder (yield 24%); m.p. 238-240 °C. ¹H NMR (400 MHz, CDCl₃-d₆) δ_H 10.21 (s, 1H, NH), 7.24-7.08 (m, 3H, Ar), 6.63 (t, 1H, Ar), 6.18 (dd, 2H, J = 7.7 Hz, 5.0 Hz, Ar), 5.04-4.81 (m, 1H, CHF), 4.44 (d, 1H, CH), 3.88-5.59 (m, 3H, CH₂), 3.58-3.32 (m, 1H, CH₂), 3.31-3.19 (m, 1H, CH₂), 2.40-2.24 (m, 1H, CH₂), 2.18-1.61 (m, 11H, CH₂) ¹³C NMR (100 MHz, CDCl₃-d₆) δ_c 171.4, 148.0, 147.7, 129.6, 122.4, 116.9, 111.1, 65.9, 58.8, 48.5, 38.8, 30.9, 23.9, 12.4. MS (ES+), [M + Na]⁺ (100) 492.2 HRMS calculated for 492.1875 C₂₆H₂₆O₂N₃F₃Na, found 492.1872; Purity HPLC 96% (Method A) R_t = 2.20 min.

Preparation of 4(R)-1-(4-(5,7-difluoro-3-methyl-4-oxo-1,4-dihydroquinolin-2-yl)phenyl)-N,N-dimethylazetidine-2-carboxamide 45f. White solid (0.056 g, 14%). δ_H [400 MHz, (CD₃)₂SO] 1.87

(3 H, s, CH₃C), 2.30-2.40, 2.60-2.75 (2 H, 2m, CCH₂C), 2.88, 2.94 (6 H, 2s, Me₂N), 3.72, 3.93 (2 H, 2m, CH₂N), 4.92 (1 H, approx. t, CHN), 6.51 (2 H, d, ArH), 7.00 (1 H, m, ArH), 7.17 (1 H, m, ArH), 7.33 (2 H, d, ArH) and 11.49 (1 H, br s, NH); δ_C [100 MHz, (CD₃)₂SO] 12.5, 22.1, 35.4, 35.8, 49.0, 63.2, 111.5, 116.3, 123.0, 129.8, 148.0, 151.9, 170.5 and 175.4; not all the aromatic carbons were seen; m/z (ES +ve mode) 398 (MH⁺, 100%); Found: m/z, 398.1667. C₂₂H₂₂N₃O₂F₂ requires m/z, 398.1680; Anal. C₂₂H₂₁N₃O₂F₂ requires C 66.49%, H 5.33%, N 10.57%, found C 66.15%, H 5.36%, N 9.88%.

Preparation of (R)-N-(tert-butyl)-1-(4-(5,7-difluoro-3-methyl-4-oxo-1,4-dihydroquinolin-2-yl)phenyl)azetidine-2-carboxamide 45g. Pale yellow powder (0.033 g, 12%). δ_H [400 MHz, CDCl₃] 1.42 (9 H, s, Me₃C), 2.00 (3 H, s, CH₃C=), 2.20-2.30 (2 H, m, CCH₂C), 3.26 (1 H, m), 3.58 (1 H, m), 3.95 (1 H, m), 6.52 (2 H, d, ArH), 6.60-6.70 (1 H, m, ArH), 7.19 (1 H, m, ArH), 7.40 (2 H, d, ArH) and 10.43 (1 H, br s, NH); m/z (CI, methane) 426 (MH⁺, base peak). Found: m/z, 426.1988. C₂₄H₂₆F₂N₃O₂ requires m/z, 426.1986; Anal. C₂₄H₂₅N₃O₂F₂ requires C 67.75%, H 5.92%, N 9.88%, found C 67.26%, H 5.88%, N 9.56%.

Preparation of 2-(4-(3,3-difluoropyrrolidin-1-yl)phenyl)-5,7-difluoro-3-methylquinolin-4-yl acetate 46. To a suspension of 2-(4-(3,3-difluoropyrrolidin-1-yl)phenyl)-5,7-difluoro-3-methylquinolin-4(1H)-one (280mg, 0.74mmol) in THF (15ml), ^tBuOK (172mg, 1.5mmol) was added. The resulting mixture was kept stirring at room temperature for 1 hour. After that, excess acetyl chloride (0.2ml) was added and the reaction mixture was kept stirring for 3 hours at room temperature. After that, H₂O (15ml) was used to quench the reaction and Et₂O (50ml) was used to dilute the mixture. Organic layer was separated from the water layer, and DCM/MeOH (1:1, 20ml) was added to the organic layer to dissolve any precipitation. The organic solution was dried with MgSO₄ and concentrated *in vacuo* to give the crude product. The crude product we purified by

flash column chromatograph eluting with 20% EtOAc in hexane to give the title product a pale yellow solid (290mg, 94%). δ_{H} [400 MHz, CDCl_3] 7.72 – 7.53 (m, 3H), 6.99 (dd, $J = 15.1, 5.7$ Hz, 1H), 6.66 (d, $J = 8.6$ Hz, 2H), 3.75 (t, $J = 13.2$ Hz, 2H), 3.61 (t, $J = 7.1$ Hz, 2H), 2.54 (ddd, $J = 21.2, 14.0, 7.3$ Hz, 2H), 2.46 (s, 3H), 2.32 (s, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 168.52, 163.55, 161.69 (dd, $J = 249.3, 14.3$ Hz), 157.00 (dd, $J = 258.3, 14.3$ Hz), 150.99 (t, $J = 1.8$ Hz), 149.05 (dd, $J = 14.2, 2.6$ Hz), 147.41, 130.57, 128.55, 128.04, 125.58, 121.90, 111.53, 109.74 (dd, $J = 20.6, 5.0$ Hz), 109.51 (dd, $J = 9.3, 1.8$ Hz), 103.05 (dd, $J = 29.3, 25.9$ Hz), 55.33 (t, $J = 31.6$ Hz), 45.54 (t, $J = 3.2$ Hz), 34.28 (t, $J = 24.0$ Hz), 20.71, 13.71; HRMS (ES) $\text{C}_{22}\text{H}_{18}\text{N}_2\text{O}_2\text{F}_3^{23}\text{Na}$ $[\text{M}+\text{Na}]^+$ requires 441.1202, found 441.1212; Anal. $\text{C}_{22}\text{H}_{18}\text{N}_2\text{O}_2\text{F}_4$ requires C 63.16%, H 4.34%, N 6.70%, found C 62.77%, H 4.29%, N 6.53%.

Biology

Drug susceptibility assays using replicating and hypoxic Mtb - For drug susceptibility assays, aerobic cultures of Mtb H37Rv were cultured as described previously ¹⁴. Cultures were grown until a mid-log growth phase was reached (Middlebrook 7H9 broth with addition of 10% albumin–dextrose–catalase solution (Becton Dickinson), 0.2% [vol/vol] glycerol and 0.05% [vol/vol] Tween 80). Hypoxic cultures of Mtb were produced using the same growth media but the method described by Wayne and Hayes was utilised ⁵⁸, where oxygen supply was limited over six weeks and cultures were mixed using 8-mm Teflon-coated magnetic stirring bars (120 rpm, 37°C).

The effectiveness of test drugs to prevent Mtb growth was determined using a microplate AlamarBlue assay (MABA) as described previously ¹⁴. A range of test drug concentrations (10 μM to 0.08 μM , 2% DMSO) were co-incubated with replicating Mtb (OD 0.01, 7 days, 37°C) followed by a MABA. Measurements of well absorbance at 570 and 600 nm recorded using an Opsys MR plate reader were determined to calculate IC_{50} values for the inhibitors. For anaerobic

cultures, co-incubations of hypoxic Mtb and test drug were performed as described for replicating Mtb, however the plates were sealed within GasPak EZ pouches containing an indicator to ensure anaerobic conditions were maintained. The plates were subsequently incubated anaerobically (7 days, 37°C) before being moved to an aerobic environment for a further 7 days. The IC₅₀ values were calculated as described for aerobic cultures.

***In vitro* Metabolic Stability** - Mixed pools of microsomes from multiple donors were purchased from BD Biosciences, USA (Human, Rat and Mouse) (protein content 20 mg/mL). Compounds of interest were tested at 10, 1 and 0.1 µM with a final concentration of microsomal protein of 1 mg/mL. The reaction was initiated by the addition of NADPH (1 mM) and samples were incubated for up to 60 min at 37°C in a shaking incubator. The reaction was terminated at 0, 10, 30 and 60 min by the addition of ice cold ACN/MeOH (50:50) spiked with internal standard. Sample preparation for mass spectrometry involved the addition of an equivalent amount of water to each sample before extraction using ethyl acetate (3 x 500 µL). The organic layer was then dried under nitrogen before reconstitution in MeOH/H₂O (50:50).

Cytotoxicity assay in HEPG2 using MTT - The cellular toxicity of test compounds were determined using the MTT assay, with modifications, using HEPG2 cells which were either resistant (cultured using glucose-containing media) or susceptible (cultured using galactose-containing media) to mitochondrial-toxicity-induced cell death^{59,60}. Briefly, HepG2 cells cultured in glucose media (high-glucose Dulbecco's modified Eagle's medium (DMEM) containing 25 mM glucose and 1 mM sodium pyruvate, supplemented with 5 mM HEPES, 10% [vol/vol] fetal bovine serum (FBS), and 100 µg/ml penicillin-streptomycin) or galactose media (glucose-free DMEM supplemented with 10 mM galactose, 5 mM HEPES, 10 % [vol/vol] FBS, 1 mM sodium pyruvate,

and 100 µg/ml penicillin-streptomycin) were added to 96-well plates (60 µl, 1×10^4 cells/well) and incubated for 24 hours. Log-range concentrations of each test compound (1-100 µM) were then added to the plates and a further incubation of 24 hours performed. Plates were subsequently incubated for 2 hours in the presence 1 mg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution. Cell lysis solution (50 µL, 50% [vol/vol] dimethylformamide in distilled water, 20 % [wt/vol] sodium dodecyl sulphate) was added to wells and plates were wrapped in metallic foil and mixed at 60 rpm for 2 hours at room temperature. Well absorbance at 560 nm was determined using a Varioskan plate reader (ThermoScientific) and were used to determine IC₅₀ values using a four parameter logistic function using Prism 5 software. All incubations were performed at 37 °C in a CO₂ incubator and compounds were solubilised in DMSO (1% [vol/vol] final concentration). The cytotoxic control compounds rotenone (0.001 µM – 1 µM, toxic to mitochondria) and tamoxifen (1-100 µM, no specific mitochondrial toxicity) were included as controls, as was a drug-free control containing 1% [vol/vol] DMSO.

Caco-2 transepithelial drug transport - Caco-2 monolayer experiments were performed as previously described ⁶¹, with modifications. When confluent, Caco-2 cells were seeded onto polycarbonate membrane transwells at a density of 2.6×10^5 cells/cm² (DMEM, 15% [vol/vol] FCS) and incubated (37°C, 5% CO₂) for 16 hours. Following this incubation, media was replaced to remove dead cells and to prevent the formation of multiple layers of cells settling on the filter. Plate media was changed every 48 hours and plates used in experiments 21 days from initial seeding. Monolayer integrity was checked using a MillicellERS instrument (Millipore) to determine the trans-epithelial electrical resistance (TEER) across the monolayer. A TEER of more than 400 Ω/cm² was deemed acceptable.

On the day of the experiment, the TEER was assessed and the media replaced with warm transport buffer (HBSS, 25 mM HEPES, 0.1% [wt/vol] bovine serum albumin, pH 7) and allowed to equilibrate (37°C, 30 minutes). The transport buffer in the chambers was replaced with transport buffer containing either the test compound or the control drug verapamil (5 µM). Samples (50 µL) were taken from the receiver compartment at 0, 60, 120 and 180 minutes and replaced with an equal volume of transport buffer. Samples were analysed using LC-MS/MS. Data were used to determine apparent permeability (P_{app} , 10^{-6} cm/s) for each direction and efflux ratio (ratio of basolateral to apical P_{app} compared with apical to basolateral P_{app}). P_{app} was calculated using the following equation as described previously ⁶²:

$$P_{app} = \frac{(dQ / dt) \times V}{A \times C_0}$$

dQ / dt is the change in drug concentration in the receiver chamber over time (nM/s); V is the volume in the receiver compartment (mL); A is the total surface area of the transwell membrane (cm^2); C_0 is the initial drug concentration in the donor compartment (nM); and P_{app} is the apparent permeability ($\times 10^{-6}$ cm/s).

Plasma protein binding using equilibrium dialysis - The extent of plasma protein binding for each test compounds was determined by equilibrium dialysis. Test compound was added to human plasma which was mixed and heated (1 µM, 1% [vol/vol] DMSO, 37°C). Regenerated cellulose membranes (5000 Daltons, Harvard Apparatus) were soaked in phosphate buffer for 5 minutes and placed within Fast Micro-Equilibrium Dialyzers (Harvard Apparatus). One millilitre plasma containing the test drug was added to the first compartment, and 1 mL phosphate buffer (1% [vol/vol] DMSO, 37°C) was added to the second compartment. Equilibrium dialysis was

undertaken by incubation (18 hours, 37°C) and samples were removed from each compartment for LC-MS/MS analysis.

Plasma Stability - Compounds were incubated in rat or human plasma (1 µM) at 37 °C for up to 3 h. At various time-points (0, 10, 30, 60, 120 and 180 min) an aliquot (100 µL) was taken and the reaction was terminated by the addition of ice cold ACN/MeOH (300 µL, 50%:50% [vol/vol]) spiked with internal standard. Samples underwent centrifugation to remove the protein precipitate and were analysed directly using LC-MS/MS analysis.

In vitro CYP450 Inhibition - CYP450 VIVID® inhibition kits were purchased from Invitrogen Life Technologies™. Briefly, compounds were tested at a final concentration of 10, 1 and 0.1 µM alongside a relevant positive control for the isoform of interest and a solvent control. The assay utilised a substrate, specific to the isoform, which produced a fluorescent metabolite as it underwent oxidation by the P450 enzyme. Inhibition of the enzyme led to reduced fluorescent output. The assay was carried out in kinetics mode, with a reading being taken every minute for a total of 1 h.

Pharmacokinetic Studies in Rats - Male Wistar rats (180 – 250 g) (n=4) were purchased from Charles River Laboratories, UK and allowed to acclimatise for 1 week in controlled conditions (23 ± 3 °C; relative humidity 50 ± 10 %; light-dark cycle 12 h). Animals were provided with feed pellet and filtered water *ad libitum*. Each rat received an oral dose of the relevant compound (10 or 50 mg/kg) in PEG400 (100 %) (5 mL/kg) via gavage needle or an IV injection of the relevant compound (0.5 mg/kg) in 5% PEG400 and 5% Solutol in water. At various time-points the rats were anaesthetised using isoflurane and a blood sample (< 300 µL) was taken from a superficial vein in the tail. The blood was immediately stored on ice before undergoing centrifugation at 13,000 rpm, for 10 minutes. An aliquot of 100 µL plasma was removed and added to ACN/MeOH

(300 µL, 50%:50% [vol/vol]) spiked with internal standard. Samples were then analysed using LC-MS/MS within 24 hours of obtaining the final sample.

PK data were modelled using the package Pmetrics®⁶³ utilising a one compartment gut absorption model. Separate doses were modelled separately to differentiate the effect of dose upon the pharmacokinetic profile of each compound.

LC-MS/MS - Drug concentration analyses were performed on a TSQ Quantum Access mass spectrometer (Thermo, UK). Chromatographic separation for all test compounds and control compounds was performed at 30°C on a Fortis C-18 3 µm column (50 X 2.1 mm i.d., Fortis technologies, UK). Mobile phases were solution A (100% acetonitrile) and solution B (100% LC-MS/MS-grade water, 0.05% formic acid) and flow rate was 0.3 mL/min. Separation was achieved with a gradient elution beginning with 90% solution B and 10% solution A, which was maintained for 1 minute. Solution A was then gradually increased to 80% over 1.9 minutes and maintained for a further 1.4 minutes. Solution B was increased to 90% over 0.7 minutes and maintained for 0.2 minutes, giving a total run time of 5.2 minutes. Robustness of analyses were assessed using standard concentration curves and quality control concentrations, where concentration standard deviations were required to be within 20% for generated results to be accepted.

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Notes

The authors declare no competing financial interest.

ASSOCIATED CONTENT

Supporting Information.

Supporting information includes:

- (i) Quinolone screening summary
- (ii) Full experimental for all intermediates.
- (iii) Metabolite identification report for MTC420.
- (iv) Molecular formula strings.

This material is available free of charge via the Internet at <http://pubs.acs.org>.

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ABBREVIATIONS

TB – tuberculosis, MDR – multi-drug resistant, XDR – extensively drug resistant, Mtb – *Mycobacterium tuberculosis*, NADH - Nicotinamide adenine dinucleotide, ETC – electron transport chain, ATP - Adenosine triphosphate, ETF – electron transferring flavoprotein, FRD – fumarate reductase, nar – nitrate reductase, HTS – high throughput screen, DMPK – drug metabolism and pharmacokinetics, SAR – structure activity relationship, DMF – dimethyl formamide, GSK – Glaxosmithkline, NBS – *N*-bromo succinamide, DCM – dichloromethane, PCC - pyridinium chlorochromate, EDC -1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide, NHS – *N*-hydroxy succinamide, GLU – glucose, PPB – plasma protein binding, CL - clearance, AUC- area under the curve, TI – therapeutic index, hERG - human Ether-à-go-go-Related Gene, NC – not calculated, ND – not determined, ID – identification, M – metabolite, SD – Sprague Dawley, HPLC – High performance liquid chromatography, TLC – thin layer chromatography, DMSO – dimethyl sulfoxide, NADPH - nicotinamide adenine dinucleotide phosphate, MTT - 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, DMEM - Dulbecco's Modified Eagle's Medium, FBS – fetal bovine serum, HEPES - (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, FCS – fetal calf serum, TEER - trans-epithelial electrical resistance, HBSS – Hank's balance salt solution, LC-MS – Liquid chromatograph-mass spectrometry.

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